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CHROMOSOMAL VARIATIONS CORRELATED WITH GEOGRAPHICAL DISTRIBUTION IN *CIRCOTETTIX* *VERRUCULATUS* (ORTHOPTERA)

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TWO TEXT FIGURES, TWO CHARTS, AND NINE PLATES

AUTHOR'S ABSTRACT

The first spermatocytes of *Circotettix verruculatus* have eleven chromosomes. Five of these are regularly atelomitic and three are telomitic. The other three are variable and may have both diads telomitic, both atelomitic, or one diad telomitic while the other is atelomitic. The locus of fiber attachment and, consequently, the form of the chromosome are constant for the individual. The point of fiber insertion is known to be inherited according to mendelian principles, and is, therefore, a measure of the frequencies of the telomitic and atelomitic diads in a group of individuals.

Samples of five geographically different populations were studied with respect to the proportion of atelomitic to telomitic diads in the three variable tetrads. The proportion for each of the three chromosomes was compared with that for the corresponding tetrads from the other localities. The data were subjected to statistical analysis, and significant differences between some of the groups were found in the proportion of atelomitic to telomitic diads in corresponding chromosomes.

A possible correlation between these cytological differences among the various localities and the formation of geographical races or subspecies are discussed. An inversion of the portion of a chromosome, which causes an apparent, although not a real, change in the locus of fiber attachment, is suggested as the origin of atelomitic chromosomes. The effect which heteromorphism of synaptic mates may have on crossing-over is considered.

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INTRODUCTION

1. Statement of problem

In a study of the relative proportions between chromosomes with atelomitic (non-terminal) and those with telomitic (terminal) fiber attachments, Carothers ('17) found that individuals of *Trimerotropis fallax* from widely separated localities arranged themselves into two groups. She found that the individuals in one group had many more atelomitic chromosomes than those in the other. Mr. A. G. Rehn, of the Academy of Natural Sciences, Philadelphia, without any knowledge of the chromosomal characters further than that they indicated that two distinct groups were concerned and without the locality labels of these animals, but entirely from a taxonomic study of external characters, divided them into two groups, which corresponded very closely with their chromosomal characters. At first he placed three individuals out of fifty-one members of group B in group A, when, according to their cytology, they should have shown greater affinities with group B; later, he did change them to group B.

In group A the number of atelomitic diads ranged from 9 to 15, with a mean of 12.3, while in group B the number of atelomitic diads varied from 6 to 11, with a mean of 8. These data, which are shown graphically by Carothers ('17, p. 463), demonstrated that the difference between these two groups is undoubtedly significant.

The purpose of this work was to make a more detailed study of similar chromosomal variations within a species using samples from widely separated and distinct populations.

2. Material and methods

Circotettix verruculatus, the only species of the genus that occurs in the eastern part of North America, is a member of the subfamily Oedipodinae of the orthopteran family Acrididae.

Grasshoppers of this species were collected in five localities, as follows: 1) University of Michigan Biological Station, Douglas Lake, Cheboygan County, Michigan; 2) the slopes of Mount Wachusett in the central part of Massachusetts; 3) the Uncanunic Mountains near Manchester, New Hampshire; 4) the hills flanking Mount Greylock in the extreme north-western part of Massachusetts, and, 5) various places on Mount Desert Island, Maine. Sixty-four animals in each case were studied as samples of the populations of Mount Wachusett, Mount Desert Island, and northern Michigan; seventy-three from Manchester, and thirty from Mount Greylock.

The testes were fixed in B_3 + chromic acid (three drops of a 50 per cent aqueous solution to each 5 cc. added immediately before using). The sections were cut $10\ \mu$ in thickness and the material stained with Heidenhain's iron-haematoxylin.

3. *Acknowledgments*

I wish to thank Dr. C. E. McClung for making it possible to undertake the problem and for his helpful interest in it. I am especially grateful to Dr. E. Eleanor Carothers for the suggestion of the problem, for much invaluable assistance throughout the course of the work, and for the use of much of her material. Also I am greatly indebted to Dr. Horace B. Baker for the specimens collected in Michigan and to Dr. Robert L. King for help in the statistical treatment of the data. The work was done at the Zoological Laboratory of the University of Pennsylvania and, during the summers of 1924-1925, at the Marine Biological Laboratory at Woods Hole. The writer wishes to express his appreciation to both of these institutions for laboratory facilities.

OBSERVATIONS

1. *Chromosomal conditions in the species*

Circotettix verruculatus has twenty-one chromosomes in the spermatogonia and male somatic cells. The discrepancy

between this number and twenty-three, the number of chromosomes characteristic for the Acrididae, is due to the formation of two multiple chromosomes.

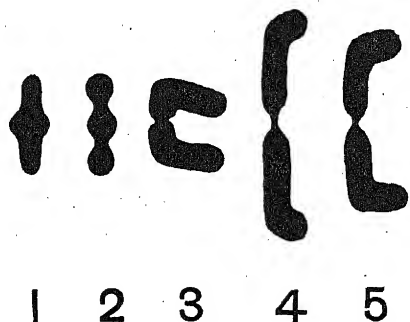
The first spermatocytes contain eleven chromosomes; nine tetrads, one octad multiple, and the accessory, which is a diad. In all the plates first spermatocytes only are shown, with the chromosomes arranged from left to right in a decreasing series according to size and numbered from 12 to 1. This arrangement is the same as was used by Carothers ('21) for this species.

The four largest chromosomes (columns 9, 10, 11, 12) and the accessory (column 6) are constantly atelomitic, so far as known. Chromosomes 2, 4, and 5 are regularly telomitic for all observed cases; in contrast with this, chromosomes 1, 7, and 8 vary from individual to individual. McNabb ('28) has shown similar conditions to occur in the first oocytes, except that the accessory chromosome is a tetrad in these cells, instead of a diad as in the first spermatocytes.

The second largest chromosome (column 11) in the complex is an octad multiple formed by the union of two tetrads. In one specimen of laboratory stock (i.e., eggs laid and hatched, and the animals raised under laboratory conditions) the octad multiple occasionally broke down into its two constituent tetrads. This unusual arrangement is shown in figure 61 (taken from Carothers ('21), pl. 2, fig. 14), in which cell the constituents of the multiple are completely separated, demonstrating that it is formed by the union of a tetrad of intermediate size and a small tetrad, which would be no. 3 in the size series. Column 3 in the plates has been left vacant for this reason. The two tetrads, composing the multiple, are themselves both telomitic. Figure 60 (Carothers' fig. 13) represents another complex from the same individual in which the octad is only broken down at one end (column 11). *Circotettix verruculatus*, then, so far as the total number of chromatids is concerned, is not an exception to other Acrididae. The multiple cannot always be distinguished with assurance from the other three large

chromosomes; but those cells in the individual just described, in which the multiple had broken down, showed that the second largest chromosome in the size series was lacking. Therefore, the multiple has been assigned to column 11.

The behavior of the three variable tetrads 1, 7, and 8 is the phenomenon of especial interest in this paper. Tetrads 7 and 8 cannot be distinguished from each other as to size; but, when atelomitic, tetrad 8 has approximately median fiber attachments, while tetrad 7 has subterminal fiber inser-



Text fig. A Drawings of tetrads 1, 2, 7, and 8 from first spermatocytes of *Circotettix verruculatus*. Nos. 1 and 2 represent tetrads 1 and 2, respectively, from the same cell and show the characteristic appearance of each in metaphase. Nos. 3 and 4 illustrate tetrad 7. No. 3 shows how this tetrad may occasionally divide with the long arms free. No. 5 represents tetrad 8 and shows the characteristically more median point of fiber attachment as compared to the subterminal attachment of tetrad 7 in no. 4.

tions. A comparison of 4 and 5 in text figure A shows this difference in loci of fiber attachments for these two tetrads. Tetrad 7 quite frequently divides, as shown in 3, where the longer arms of both homologues are free, thereby giving this tetrad an unmistakable appearance when it opens out in this way. When both tetrad 7 and 8 are telomitic, they cannot be distinguished; but in such cases their separation is not necessary, since both are alike. However, the condition in which both tetrads are telomitic occurs only very infrequently, as tetrad 7 has one or both diads atelomitic, on an average, in 95 per cent of the cases.

Tetrad 1 is the smallest chromosome in the complex, but is so nearly the size of tetrad 2 that this single criterion cannot be used for their certain differentiation. However, in the late metaphase, the distal ends of the diads of tetrad 2 are swollen; this difference in appearance from tetrad 1 is shown in text figure A, where nos. 1 and 2 represent these two chromosomes drawn from the same cell. Although this difference in shape tends to disappear as the degree of separation of the diads increases, cells can always be found in each testis that are in the late metaphase. Chromosome 2 has never shown any tendency to become atelomitic in this species, though King ('23) has found it to be so in two species of a closely related genus.

Each individual is constant as regards the locus of fiber attachment and, consequently, the form of the chromosomes. Carothers ('21) has shown that offspring have in their cells only such chromosome shapes as are contained in those of their parents, and these shapes are arranged in only those combinations which would be expected on mendelian principles. The mode of fiber insertion of each chromosome is inherited independently of that of any other chromosome in the complex. An examination of chart 1 will corroborate her results, although mine are arrived at in a different fashion.

The frequencies of atelomitic fiber attachments in tetrad 1 were plotted from left to right according to the increasing proportions of this condition in the five populations.

In chart 1 the order of populations established for tetrad 1 was maintained for tetrads 7 and 8, and a comparison of the frequency of atelomitic fiber insertions in tetrad 1 for any one group with that of tetrads 7 and 8 in individuals from the same locality will, thus, show no correlation between the frequency of this condition in one tetrad and its occurrence in the other two tetrads of the same group.

2. Method of statistical analysis

The dividing tetrads of the first spermatocytes show very clearly whether their constituent diads are telomitic or

atelomitic and can be used to determine the frequencies of these conditions in the various populations. The numbers of atelomitic diads in the three variable tetrads 1, 7, and 8 were recorded for each individual from the five localities. These data were subjected to statistical analysis to ascertain

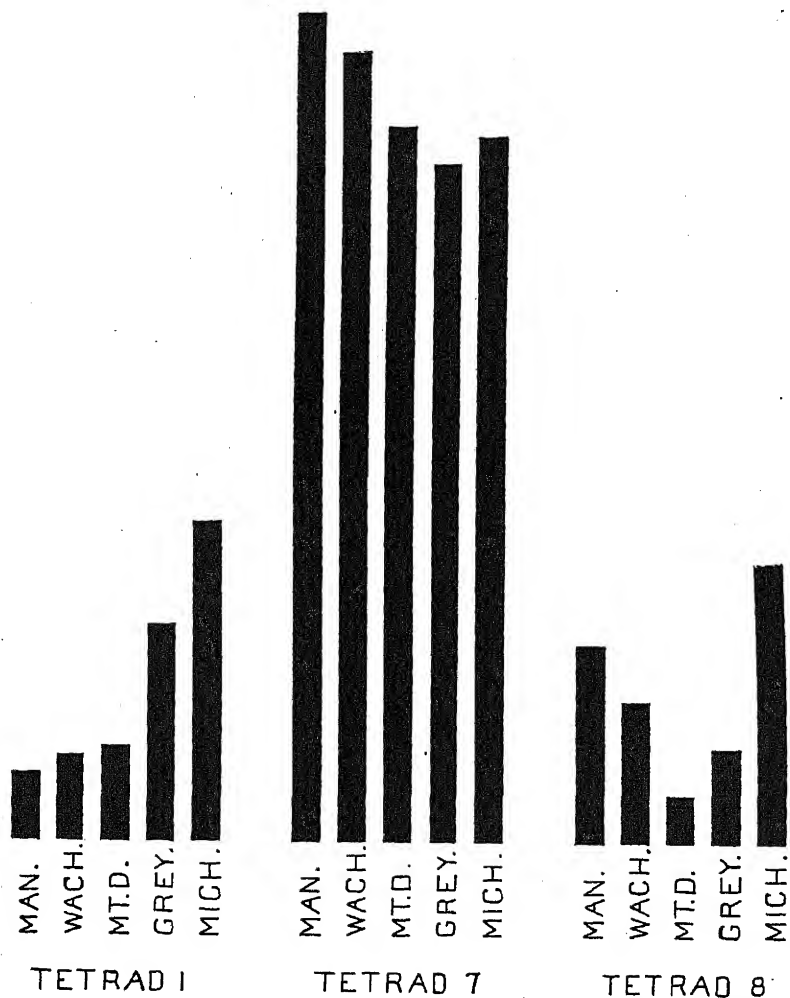


Chart 1 Relative frequencies of atelomitic diads in tetrads 1, 7, and 8 for the various localities.

whether the differences between the diverse populations, with respect to these variable tetrads, were of sufficient magnitude to be significant.

Table 1 shows the observed and calculated frequencies of telomitic and atelomitic diads in the three tetrads for all groups. Use is made of the data concerning tetrad 1 from

TABLE 1

Comparison between the observed and calculated frequencies of telomitic, heteromorphic, and atelomitic conditions in tetrads 1, 7, and 8 for the various localities

LOCALITY	CONDITION OF TETRAD	TETRAD 1		TETRAD 7		TETRAD 8	
		Observed	Calculated	Observed	Calculated	Observed	Calculated
Greylock	Telomitic	17	17.6	3	2.4	25	22.6
	Heteromorphic	12	10.7	11	12.2	4	6.9
	Atelomitic	1	1.7	16	15.4	1	0.5
Manchester	Telomitic	64	63.4	1	1.1	46	45.3
	Heteromorphic	8	9.3	16	15.8	23	24.4
	Atelomitic	1	0.3	56	56.1	4	3.3
Michigan	Telomitic	29	27.5	4	4.0	34	30.9
	Heteromorphic	26	28.9	24	24.0	21	27.2
	Atelomitic	9	7.6	36	36.0	9	5.9
Mount Desert Island	Telomitic	51	51.6	5	3.8	58	58.0
	Heteromorphic	13	11.7	21	23.5	6	5.8
	Atelomitic	0	0.7	38	36.7	0	0.2
Wachusett	Telomitic	54	53.5	0	1.7	48	46.5
	Heteromorphic	9	10.0	21	17.5	13	16.1
	Atelomitic	1	0.5	43	44.8	3	1.4

the Greylock material to demonstrate how the theoretical distribution of telomitic and atelomitic diads was computed for the five groups. The observed frequencies in the thirty individuals of this population show that fourteen diads (23.4 per cent of the total sixty diads) were atelomitic, while forty-six (76.6 per cent) were telomitic. Then the probability of obtaining simultaneously by random sampling two atelomitic diads, two telomitic diads, or one atelomitic and one telomitic

diad was calculated. The probability of the occurrence together of two independent events is the product of their separate probabilities. Therefore, the probability of the simultaneous occurrence of two atelomitic diads in this group of thirty individuals is $(.234)^2 \times 30 = 1.7$ as compared with the observed value of 1. In a similar manner, the chance of the occurrence together of two telomitic diads or of one telomitic diad and one atelomitic diad was calculated. Similar calculations were made for all the groups, and these data are summarized in table 1.

This table shows that the actually observed occurrences of homomorphic telomitic, heteromorphic, and homomorphic atelomitic tetrads fit very closely in all cases the theoretical expectations as calculated for each class on the basis of the observed frequencies of telomitic and atelomitic diads. For this reason, table 1 also demonstrates that the individuals studied were sufficiently numerous to be fair samples of the population of each locality.

The Chi Square method (Yule, '24) was used for testing whether these differences were such as would occur in random samples from a population of which the characteristics were known only from the samples or whether the probability of the relative frequencies in any one locality was really different from that of the others to such a degree that this diversity could not reasonably be supposed to have arisen from the operation of chance alone.

Table 2 shows the relative numbers of telomitic and atelomitic diads in tetrad 7 for all the localities. In this table *o* is the observed number or frequency and *c* is the calculated. As an example of how Chi Square was computed, the data in this table will be utilized. The assumption made in all cases was that the combined material from all localities was homogeneous or that the real proportion of telomitic and atelomitic diads was the same. The total number of atelomitic and telomitic diads in all the groups was ascertained, and from this the proportion of atelomitic (79.8 per cent) to telomitic (20.2 per cent) diads was computed. From this

proportion the calculated frequency for all localities was found as previously described.

TABLE 2

Frequencies of telomitic and atelomitic diads in tetrad 7 for all localities

LOCALITY	FREQUENCIES	NUMBER OF ATELOMITIC DIADS	NUMBER OF TELOMITIC DIADS	TOTAL NUMBER OF DIADS
Greylock	o-observed	43	17	60
	c-calculated	47.9	12.1	
	o-c	-4.9	4.9	
	$\frac{(o-c)^2}{c}$.52	1.99	
Manchester	o-observed	128	18	146
	c-calculated	116.5	29.5	
	o-c	11.5	-11.5	
	$\frac{(o-c)^2}{c}$	1.13	4.48	
Michigan	o-observed	96	32	128
	c-calculated	102.2	25.8	
	o-c	-6.2	6.2	
	$\frac{(o-c)^2}{c}$.38	1.49	
Mount Desert Island	o-observed	97	31	128
	c-calculated	102.2	25.8	
	o-c	-5.2	5.2	
	$\frac{(o-c)^2}{c}$.26	1.05	
Wachusett	o-observed	107	21	128
	c-calculated	102.2	25.8	
	o-c	4.8	-4.8	
	$\frac{(o-c)^2}{c}$.23	.89	
Total number of diads		471	119	590

Sum of quantities $\frac{(o-c)^2}{c} = .0148$, or 15 cases in 1000.

Using the Greylock data, the calculated frequency of atelomitic diads ($c = 47.9$) was subtracted from the observed frequency ($o = 43$) to give the deviation ($o - c = -4.9$). This

deviation was squared and divided by the calculated number. ($\frac{(o-c)^2}{c} = 52$) This was done for all groups and the sum of these quantities was obtained, and Elderton's table (Pearson, '24) was consulted. Here it was found that the probability (P) was 0.0148 or, in other words, that one would expect to find about fifteen cases in a thousand in which as great or greater deviation might occur, provided the material was homogeneous or that the true proportion of telomitic and atelomitic diads was the same. If the probability that the material was homogeneous had been less, the data on the population, which deviated the most, would have been eliminated and the material further tested until all groups which showed deviation were disclosed. The limit of probability usually used is one case in a thousand. This limit is so high that any differences which meet its requirements can be accepted without doubt.

The differences in the proportion of the telomitic to atelomitic diads in tetrad 7 between the various groups were only such as would be expected from random sampling within the same population and, consequently, of no significance. Chart 1 shows graphically this similarity in the number of atelomitic diads in this tetrad for the various groups.

3. *Comparative frequencies of the telomitic, atelomitic, and heteromorphic conditions for corresponding tetrads in the various localities*

These several groups were made up of individuals each of which had three chromosomes with observable variations, which were of an alternative nature; i.e., each diad could have a telomitic or an atelomitic fiber attachment. As first spermatocytes were studied, this condition was reflected in the three tetrads where both homologues might be telomitic, atelomitic, or one homologue might be telomitic while the other was atelomitic, resulting in a heteromorphic tetrad.

The relative proportions of these three conditions depended upon the numbers of telomitic and atelomitic chromosomes present in the population. The frequency of the atelomitic

condition varied in corresponding tetrads from one locality to another, and, consequently, the relative proportions of telomitic and heteromorphic tetrads varied, as would be expected. Chart 2 shows the relative proportions of the telomitic, atelomitic, and heteromorphic diads in corresponding tetrads for all five localities, and these proportions in all cases approximate so closely the theoretical expectations, as shown by table 1, that nothing unusual was disclosed by the differential frequencies of these conditions in the same tetrads from various localities.

4. Chromosomal conditions in the various populations

Michigan. Plates 1, 2, and 3, figures 1 to 20, inclusive, show the different types of chromosomal complexes, and the number at the right of each figure refers to the number of times that complex occurred in the sixty-four individuals studied.

Atelomitic diads in the tetrads 1 and 8 of this group were conspicuously more frequent for these two chromosomes than for those of any other group. This is shown graphically in chart 1, which gives the relative frequencies of atelomitic diads for all groups.

Carothers ('17) and King ('23), working on several species of other genera closely related to *Circotettix* and indigenous to the western part of North America, found that not only were more than eight chromosomes involved in the shift of fiber attachment, but, also, for any one variable chromosome the frequency of atelomitics was much greater than in any of the variables (1, 7, and 8) of *Circotettix verruculatus*. King ('23) found that in *Pseudotrimerotropis thalassica* (= *Trimerotropis thalassica*) every chromosome could have an atelomitic fiber attachment. It is interesting to note that, in *Circotettix verruculatus*, the group which has the greatest number of atelomitic chromosomes is from Michigan, the most western region considered, thereby giving an intermediate condition between these western populations of closely related species and the eastern ones, where there are relatively very few atelomitic chromosomes. It is suspected

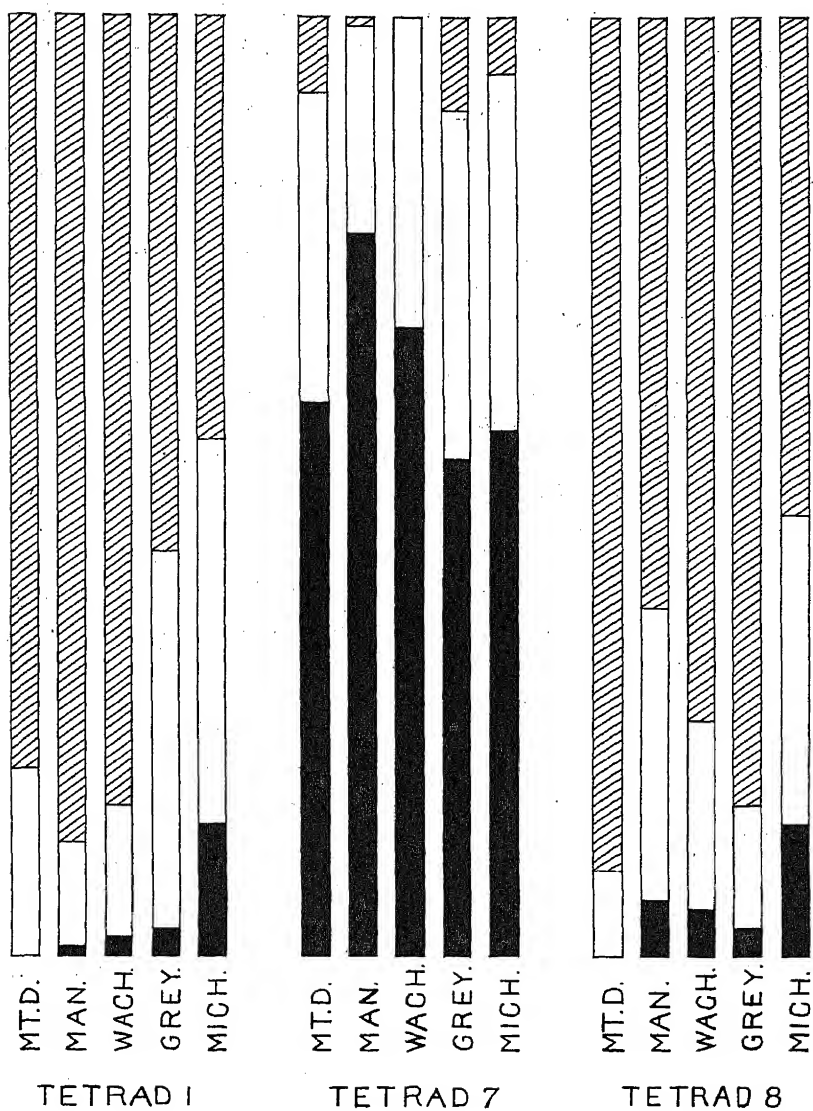


Chart 2 Relative proportions of atelomitic, heteromorphic, and telomitic conditions in tetrads 1, 7, and 8 for the various populations. The proportion of atelomitic tetrads is represented by the solid areas; the heteromorphic tetrads, by the clear areas; and the telomitic tetrads, by the cross-hatched areas.

that in this species, a more western population than Michigan would disclose a yet greater percentage of atelomitic chromosomes.

The proportion of atelomitic diads in tetrad 7 did not differ significantly from that of any other group, although it was slightly less than in three of the eastern groups, as is shown in chart 1.

The much greater percentage of atelomitic diads in both tetrads 1 and 8 rendered this group significantly different from the other four groups with respect to these two chromosomes.

Greylock. Plates 3, 4, and 5, figures 21 to 29, inclusive, give the various kinds of chromosomal combinations characteristic of this group and also the frequency with which they were encountered. Chart 1 shows the frequency of atelomitic diads in tetrads 1, 7, and 8 as compared with the frequencies of this condition for corresponding tetrads in other places. This population showed a tendency for tetrads 1 and 7 to approach to the conditions found in the Michigan material.

Tetrad 7 parallels closely that for the Michigan group, as is shown by chart 1, but, like that group, was not significantly different from those in the other populations.

Tetrad 1 had one or both of its homologues so frequently atelomitic as to indicate that for a large proportion of its population chromosome 1 was atelomitic. The frequency of this condition for chromosome 1 was so much greater than that of the corresponding chromosome in the other eastern populations as to render it significantly different from them as a group. Also, it deviated significantly from the Michigan material in possessing relatively fewer atelomitic chromosomes. With respect to chromosome 1, this population was intermediate between the other eastern populations and the western one. However, it did not differ significantly from the Michigan group nor from any of the other eastern groups with the exception of the Manchester and Wachusett material.

The frequency of atelomitic diads in tetrad 8 was significantly less than in the Michigan group, approximated that of

the population on Mount Desert Island and, like it, was significantly less than that of the Manchester and Wachusett groups.

Manchester and Wachusett. Plates 5 and 6, figures 30 to 39, inclusive, and plates 6, 7, and 8, figures 40 to 50, inclusive, show the various types of chromosomal complexes for Manchester and Wachusett, respectively, with their frequency indicated to the right of the figures. These two populations were so similar in all details, as an examination of chart 1 will show, that it seemed unnecessary to consider them separately. This is what would be expected when there are no isolating barriers. The terrain over the short distance intervening between these localities is of a similar nature to that in the localities.

In these two localities nearly half of the specimens, or 42 per cent of the Manchester and 44 per cent of the Wachusett individuals, have but one type of chromosomal complex. This complex is the same in both cases and is shown by figure 30 for Manchester and figure 40 for Wachusett.

The occurrence of atelomitic fiber attachments for tetrad 1 was very rare in both these populations, being only 1.7 per cent of the whole group for Wachusett and 1.3 per cent for Manchester. This percentage is so small as to render these groups significantly different from populations of Greylock and Michigan, though not from that of Mount Desert Island, where 1.9 per cent of the population had this chromosome atelomitic.

The proportion of atelomitic fiber attachments for tetrad 7 was higher for both these populations than for any of the others, but not significantly so.

Conditions in tetrad 8 were parallel for these two populations and approximated those of Greylock. The number of atelomitic diads was significantly less than that of the Michigan group and yet frequent enough to distinguish this material to a significant degree from that on Mount Desert Island.

Mount Desert Island. Plates 8 and 9, figures 51 to 59, inclusive, present the kinds of chromosomal complexes with their frequency of occurrence in this population. Chart 1 shows the proportion of atelomitics for the three variable chromosomes of this group. This population behaved similarly in some respects to those of Manchester and Wachusett, as one type of chromosomal complex occurred in 44 per cent of this material. This complex is similar in both type and frequency to that which is found in both the Manchester and Wachusett material.

Conditions in tetrad 1 were similar to those in the Manchester and Wachusett material. It had so few of its homologues atelomitic that it was found to be significantly different from the Michigan group.

The frequency of atelomitic diads in tetrad 7 closely approximated their occurrence in the Greylock and Michigan populations, but was less than in the Manchester and Wachusett groups. However, these diversities were too small to be considered significant.

Tetrad 8 had atelomitic fiber attachments in only 0.9 per cent of the population. The scarcity of this condition made this group significantly different from all other groups, except that of Greylock, with respect to this chromosome.

The Mount Desert Island population has the least number of atelomitic chromosomes. This locality is geographically farthest removed from those western regions, where, in species closely related to *Circotettix verruculatus*, the percentage of atelomitic chromosomes is relatively very high. Also, it is interesting and probably significant that Michigan should be intermediate both in numbers of atelomitic chromosomes and geographical situation, but the explanation of this is not apparent at present.

5. Summary of observations on the variable tetrads 1, 7, and 8

By the statistical method previously described, it was found that the observed distribution or frequency of the atelomitic diads in tetrads 1, 7, and 8 were, in some cases, significantly

different from what would have been obtained by independence in random sampling from the same population, or the differences in the frequencies of telomitic and atelomitic diads in tetrads 1, 7, and 8 for the diverse localities were, in some instances, so great as to be significant. These cases will be briefly summarized for the three variable tetrads.

Tetrad 1. It was found that the diads composing tetrad 1 in the material from Michigan were more often atelomitic than in the other groups. When this condition was examined statistically, it was found to be significantly different from all other populations. Upon further examination, the diads of this tetrad in individuals from Mount Greylock were discovered to be intermediate between the Michigan and the other eastern groups, but were not significantly different from any of them, with the exception of the Manchester and Wachusett material. Individuals from the remaining localities—Mount Desert Island, Manchester, and Wachusett—varied slightly among themselves in the relative numbers of atelomitic chromosomes in their populations, but not sufficiently to show significant diversity. Thus, the Michigan material exceeded the material from all other groups in the numbers of atelomitic diads. The Greylock group was differentiated from this material by a paucity of atelomitic diads, but did not differ significantly by their frequency from the remaining New England populations, with the exception of the Manchester and Wachusett groups, forming, consequently, an intermediate condition between the Michigan group and the groups from Wachusett, Manchester, and Mount Desert Island.

Tetrad 7. The proportion of atelomitic diads in this tetrad from all localities was such as might have been obtained by random sampling within the same population. However, the distribution of atelomitic diads was not identical in all groups, but the diversities exhibited were not of sufficient size to meet the limit of probability.

Tetrad 8. The number of atelomitic diads was significantly greater in the Michigan material than in any of the New

England populations. The Mount Desert Island material had significantly fewer atelomitic diads than that from any other locality, except Greylock.

It is interesting to note that, with respect to chromosome 1, the Greylock and Michigan materials tended to vary in the same way. In both populations the occurrence of atelomitic fiber attachments in chromosome 1 was numerous. However, as to chromosome 8, the Greylock and Mount Desert Island groups showed a tendency in the same direction, as, in both, the number of atelomitic chromosomes no. 8 was greatly diminished.

The similarity between the Wachusett and Manchester populations in the total number of atelomitic fiber attachments and the frequency of this condition in the three chromosomes was according to expectation, and the reason for this has been previously suggested.

DISCUSSION

1. Distribution of Circotettix and the formation of geographical races

Circotettix occurs generally in the northern part of North America from the Pacific to the Atlantic Oceans. It extends farther south in mountainous than in lower country, especially in the West, where it follows the ranges of the Rocky Mountains as far south as Mexico. Within these western species of *Circotettix*, Rehn ('21) has defined geographical races or subspecies. Due to the close dependence of these subspecies on their special habitat, the irregular nature of the western terrain offers many isolating barriers and thus provides ample opportunity for the establishment of such races.

Massachusetts¹ is the southern limit in the distribution of *Circotettix verruculatus*, the only species found in the eastern

¹ Individuals of *Circotettix verruculatus* have been taken as far south as the Delaware Water Gap and Dover, which are in the mountainous part of northern New Jersey. However, the presence of this species in any abundance this far south is very doubtful.

part of North America, and here it occurs only in mountainous regions at higher altitudes. The population in the Berkshire Mountains around Mount Greylock is isolated by the Connecticut River valley from that inhabiting the mountainous country in the vicinity of Mount Wachusett and Manchester, New Hampshire. In regions farther north this species is not restricted to the elevated parts of the country, but is distributed more generally.

In the distribution of this species individuals must have migrated from the north southward to the Berkshire Mountains (Mount Greylock) and to the mountainous country around Mount Wachusett and the Uncanunic Mountains near Manchester.

If the progenitors of the population in the Berkshire Mountains (Mount Greylock) had the majority of their chromosomes no. 1 with atelomitic fiber attachments, these might be segregated and tend to be multiplied, so that in time it might supplant those individuals which had chromosome no. 1 telomitic. If the factors or genes in the atelomitic chromosome 1 were different, which is easily conceivable, from those in telomitic chromosome 1, a variety or race with different genetic factors than the other populations would result, and a geographical race would have been established in this locality. The population in the vicinity of Mount Greylock has chromosome 1 more often atelomitic than that of any other New England locality.

The material from Mount Desert Island has few atelomitic fiber attachments in chromosome 8. If telomitic chromosome 8 is genotypically different from chromosome 8 with atelomitic fiber insertions, there is in this locality a race which is differentiated from the other New England races by those factors in telomitic chromosome 8.

The Michigan population has both chromosomes 1 and 8 significantly more often atelomitic than any other of the groups. If the telomitic and atelomitic conditions do reflect genotypic differences, there is, then, in this locality a race distinguished from all the others by the factors or genes carried in the atelomitic chromosomes 1 and 8.

Thus, it is suggested that, perhaps, these measurable differences demonstrated in corresponding chromosomes of individuals from diverse localities might reflect the genetical dissimilarities, which are the primordia of geographical races or subspecies.

2. Chromosomal variations and geographical races

Artom ('12) has shown that the phyllopod *Artemia salina* has developed two geographical races. In Capodistria (near Trieste) and various near-by localities is found a race which produces only parthenogenetic eggs and has 84 chromosomes; in Sardinia there exists the other form, which has 42 chromosomes and reproduces sexually.

The homopterous insect *Trialeurodes vaporariorum* has two races, as shown by Schrader ('26). In the American race the unfertilized eggs develop with the haploid number or 11 chromosomes and give rise to males only. In the English race the unfertilized eggs restore the diploid number or 22 chromosomes and produce females.

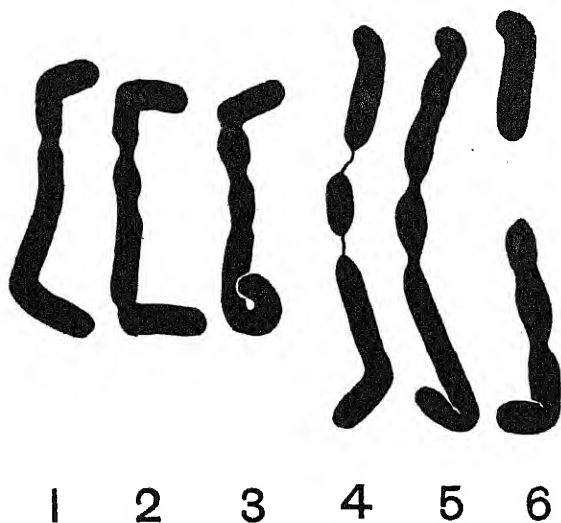
For moths of the genus *Solenobia*, Seiler ('23) has reported two species, each of which has two races differing in their mode of reproduction and geographic distribution.

There are other known cases of distinct races characterized by chromosomal differences within a species, though these are not correlated with differences in geographical distribution. For example, the two well-known races of *Ascaris megalocephala*, *univalens* and *bivalens*, with 2 and 4 chromosomes, respectively. Also, in *Parechinus microtuberculatus*, Boveri ('90, '05) has described two races in which the diploid numbers of chromosomes are 18 and 36. Bridges ('19) has reported triploid and tetraploid races of *Drosophila melanogaster*.

In the plant *Chara crinita*, Ernst ('18) has found two races; one of these is strictly parthenogenetic and diploid, while the other reproduces sexually with the haploid number of chromosomes. The diploid or parthenogenetic race undergoes no process of reduction and develops with 24 chromosomes. The

haploid sexual races produce gametes, containing 12 chromosomes, which, by their union, must produce zygotes with 24 chromosomes. However, the resulting plants have only 12 chromosomes; consequently, reduction here must be zygotic. Intermediate forms between the two races do not occur, and it is clear that they are genotypically unlike.

The previous cases of diverse races within a single species entailed a numerical difference in the chromosomes. McClung



Text fig. B First spermatocyte chromosomes of *Mermiria*-hexad multiples, showing specific differences in point of fiber attachment. Nos. 1, 2, and 3 are from *Mermiria maculipennis macelungi*; nos. 4, 5, and 6 from *Mermiria bivittata* (McClung).

('17) reported the species *Mermiria bivittata* as divisible into two groups upon the basis of a difference in the form of one chromosome. In one race both ends of a dividing hexad had the same degree of subterminal fiber attachment as shown in 1, 2, and 3, text figure B. In the other, as 4, 5, and 6, text figure B, indicates, "there is a pronounced bend at one end at the point of fiber attachment, but the other extremity is almost straight, only a slight subterminal flexure indicating the place of the other fiber insertion. In some instances this

point is almost at the end of the chromosome. Individuals with this peculiarity are clearly distinguished by somatic characters and constitute a distinct species." Rehn ('19), in a revision of this genus, described structural differences which had been overlooked before the discovery that the chromosomes of the germ cells presented these constant differences of form. These structural differences, which were correlated with the chromosomal variation described above, form the basis for the differentiation of *Mermiria bivittata* from *Mermiria maculipennis macclungi*, which had previously been confused with *bivittata*.

Blakeslee, in an address before the American Philosophical Society, Philadelphia, 1928, reported that a species of *Datura* includes races which are correlated with the number of chromosomes and the manner in which these are connected to give strings and rings. Thus, the essential number and form of the chromosomes are correlated with genetical dissimilarities.

3. Possible origin of heteromorphism and its effect on crossing-over

A terminal fiber attachment is the more usual and, probably, the primitive condition among the Acrididae. The assumption of a shift of fiber attachment at some time was made by Carothers ('17) to account for the origin of chromosomes with non-terminal or atelomitic attachments. Another possible method for the formation of atelomitic chromosomes is suggested by some recent genetical results.

Sturtevant ('26) has shown that, in a wild stock of *Drosophila melanogaster* from Prague, a portion of the right half of the third chromosome has become inverted and that this inversion acted as a cross-over reducer when heterozygous. He suggested that this was because homologous genes did not lie opposite each other, and synapsis, therefore, was not complete. Sturtevant and Plunkett ('26) have also shown the existence of an inverted section in the third chromosome of *Drosophila simulans* as compared with that of the cor-

responding chromosome in *Drosophila melanogaster*. Sturtevant suggested that some of the other cross-over reducers reported by Payne ('24) and Ward ('23) may also be due to inversions of portions of chromosomes.

Inversions of this type may conceivably be responsible for the change in fiber attachment from the more usual terminal position to that of an atelomitic or non-terminal one. Consequently, the original change may not have been a real shift in the locus of fiber insertion from the terminal position, but the result of the inversion of the distal portion of the chromosome. If the spindle fibers continued to attach themselves to the same part of the chromosome, such inversion would bring about an apparent, but not a real, change in the locus of fiber insertion. When once this change had become established, it would be perpetuated by heredity.

The change from a telomitic to atelomitic fiber insertion, whether it be by a shift in the locus of fiber attachment or by a reorganization of the chromosome, is a mutation, which must have taken place with relative frequency in some species in order to account for the large proportion of atelomitic chromosomes present, or else it must be associated with some factor of decided survival value. The change must have involved different chromosomes, for in *Circotettix verruculatus* as many as fourteen of them may be atelomitic, while in *Pseudotrimerotropis thalassica* (= *Trimerotropis thalassica*) King ('23) found that all the chromosomes may be affected. Also, the degree of the change for corresponding chromosomes in different individuals may not be the same. Carothers ('21), working on *Circotettix verruculatus*, and King ('23), for *Pseudotrimerotropis thalassica* (= *Trimerotropis thalassica*), have shown two different points of fiber insertion for corresponding chromosomes in different individuals. These cases would involve two separate inversions for each chromosome, and the length of the inverted portion of the chromosome would differ in each inversion.

The material, from which most of our knowledge of cross-over is derived, does not show heteromorphism, except

in the X-Y pair. Nevertheless, a consideration of the possible effect of this condition on crossing-over may be worth while; King ('23) has shown that the homologues of tetrad 5 in *Pseudotrimerotropis thalassica* (= *Trimerotropis thalassica*) may have either a terminal, distal subterminal or a median fiber attachment. In cases where this tetrad is made up of homologues with similar fiber attachments, that is, when they are homomorphic for telomitic, median atelomitic, or subterminal atelomitic fiber insertions, the loci of fiber attachments come to be opposite each other in synapsis, and no mechanical interference in crossing-over would result. In cases where the homologues of this tetrad were heteromorphic for fiber attachments, the loci of these insertions would not lie opposite during synapsis. If crossing-over did occur in a certain area, determined by the points of fiber insertions, one homologue with two points of fiber attachment and one with none would result, and this could not logically occur. However, double crossing-over would not be eliminated in these cases. This explanation is consistent with the genetic results, for Sturtevant ('19) has shown that many of the factors which modify crossing-over are effective only in the heterozygous condition. "In the cases of CII r and CII 1, it is to be noted that two like chromosomes cross over freely while two unlike ones do not."

Cases of failure of crossing-over in certain regions of chromosomes may not be due, as suggested by Sturtevant ('26), primarily to inversion and the resulting condition that homologous genes do not lie opposite each other, but, rather, as suggested by King ('23), to the limitations imposed upon it when one homologue is telomitic and the other is atelomitic.

CONCLUSIONS

1. The number of chromosomes in the first spermatocytes of *Circotettix verruculatus* is eleven.
2. Five of these chromosomes invariably have atelomitic fiber attachments.
3. Three chromosomes always have telomitic fiber insertions.

4. The diads, which constitute the three remaining tetrads, vary in the loci of fiber attachments from individual to individual, but they are constant within a single animal.

5. Diverse populations were studied to ascertain whether any significant diversity could be found in the proportion of these variations in fiber insertions of the three variable chromosomes.

6. In the diverse localities corresponding chromosomes give the expected proportions of telomitics, atelomitics, and heteromorphics.

7. Tetrad 7 shows no significant variation in the frequency of atelomitic fiber attachments of its constituent diads among the diverse populations.

8. The group from Michigan has a larger proportion of the diads of tetrads 1 and 8 atelomitic than that of the other localities.

9. The Greylock group shows a significantly greater proportion of atelomitic diads in tetrad 1 than do the Manchester and Wachusett groups.

10. With respect to tetrad 1, the Greylock group is intermediate between the Michigan and the remaining eastern groups in the number of atelomitic diads, but is not significantly different from either of them, with the exception of the Manchester and Wachusett groups.

11. The Greylock and Michigan materials have a greater number of atelomitic diads in tetrad 1 than do the other groups.

12. The Greylock and Mount Desert Island groups both give a very small number of atelomitic diads in tetrad 8.

13. The Manchester and Wachusett groups are comparable in every detail, as expected from the absence of barriers between these regions.

14. These differences in loci of fiber insertion and consequent chromosome shape may be correlated with genotypic dissimilarities in the chromosomes.

15. Segregation of these genotypically unlike chromosomes may account for the origin of geographical races or subspecies.

16. A possible method of origin is suggested for atelomitic chromosomes, and the effect of heteromorphism on crossing-over is discussed.

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EXPLANATION OF PLATES

The complexes were drawn with the aid of a camera lucida at a magnification of 2400 diameters. In the reproductions they appear at 1600 diameters.

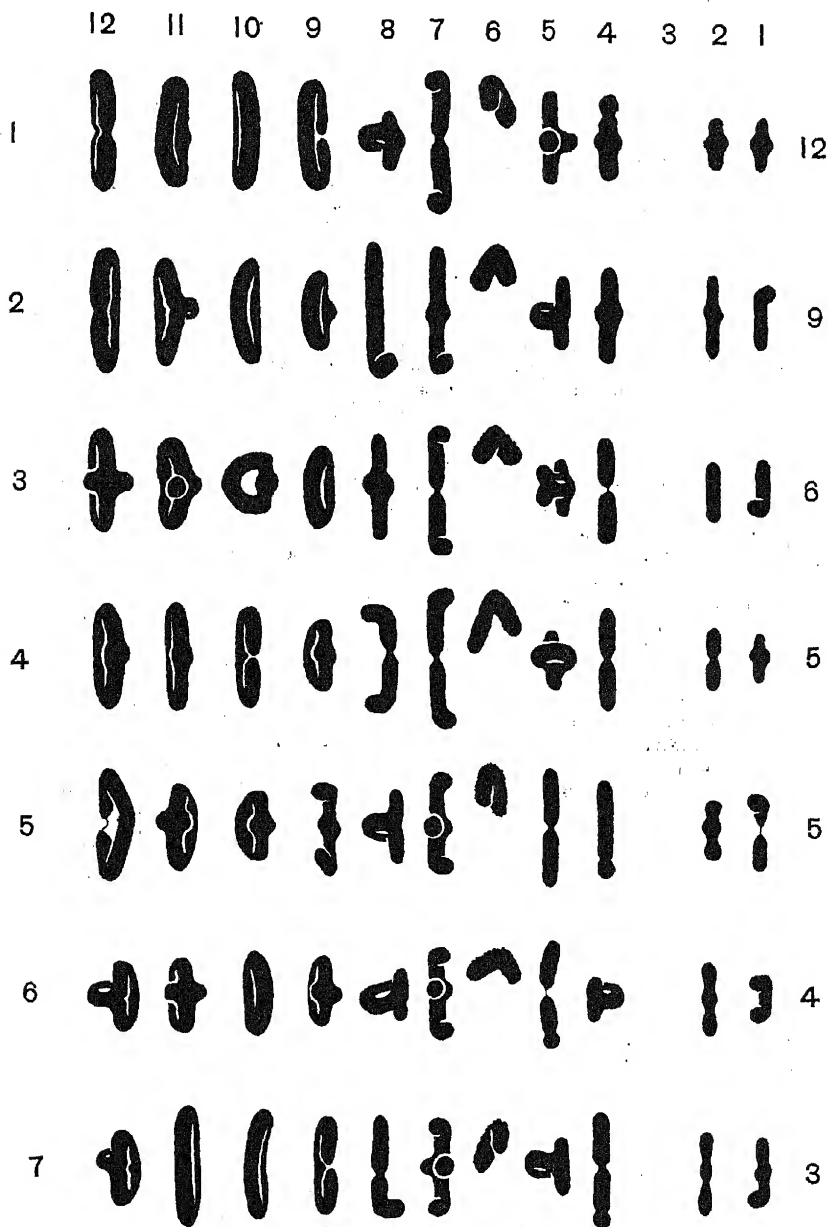
The figures are samples of all the different chromosomal combinations which are found in the various groups. The number at the right of the row refers to the number of individuals in which that type of chromosomal complex occurs in the group under consideration.

All figures are lateral views of metaphase chromosomes from first spermatocytes. The drawings of the chromosomes were rearranged approximately according to size. Each horizontal row represents the chromosomes of one cell of an individual. Each vertical column represents corresponding chromosomes from different individuals. The arrangement is such that the accessory, no. 6, is always passing to the upper pole.

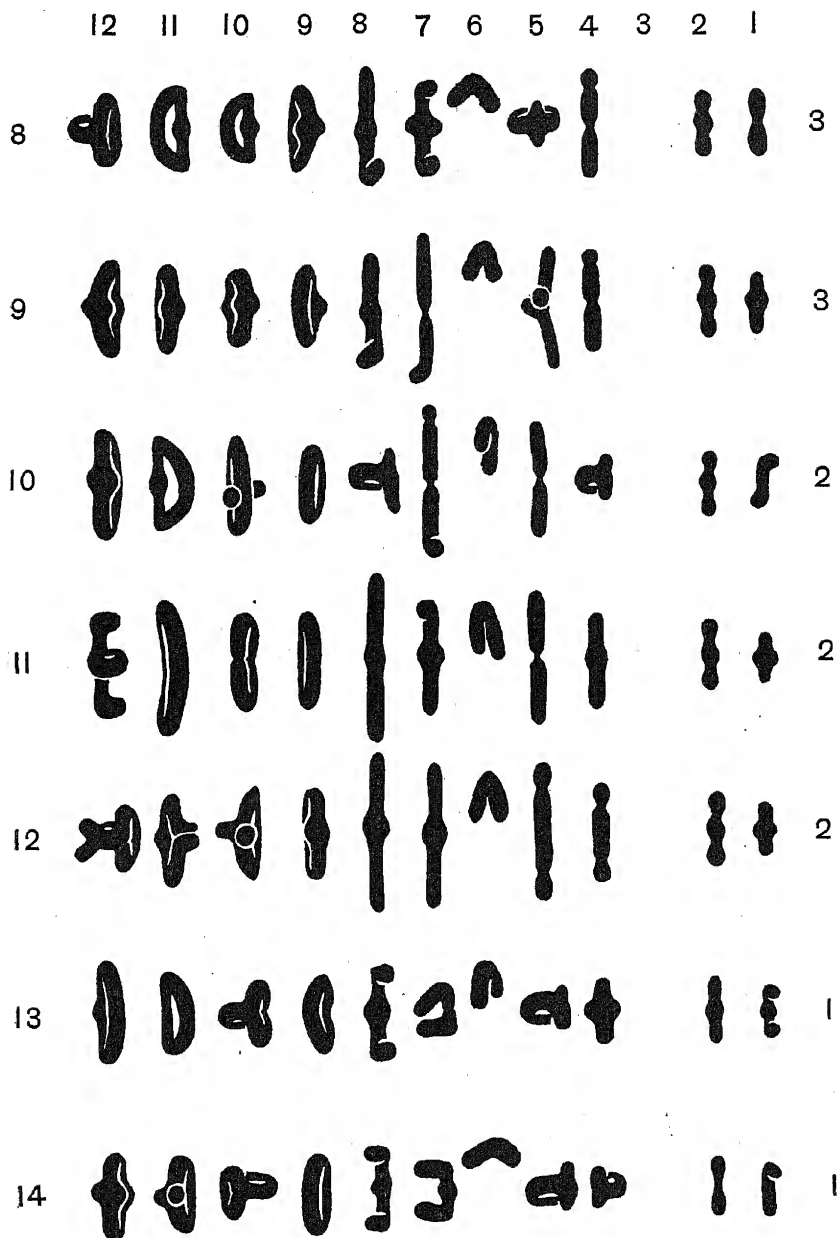
Column 3 is left vacant, since the tetrad, which belongs here in the size series, is united with another tetrad of intermediate size to form an octad multiple.

For each group the type of chromosomal complex which occurs most frequently is figured first and, so on in decreasing order, to the rarest type.

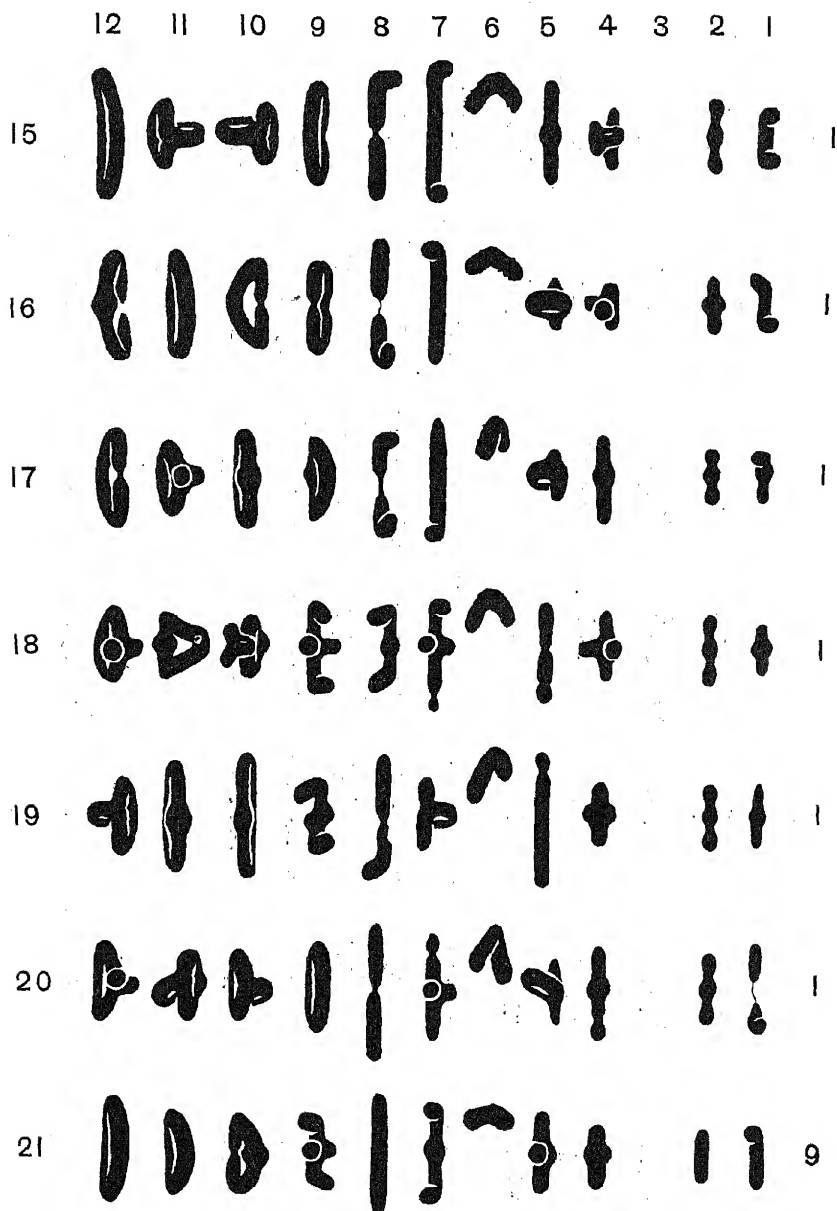
The groups are figured in order from that which shows the greatest frequency of atelomitic diads to that with the least number of them. Thus, the order is as follows: Michigan, Greylock, Manchester, Wachusett, and Mount Desert Island.



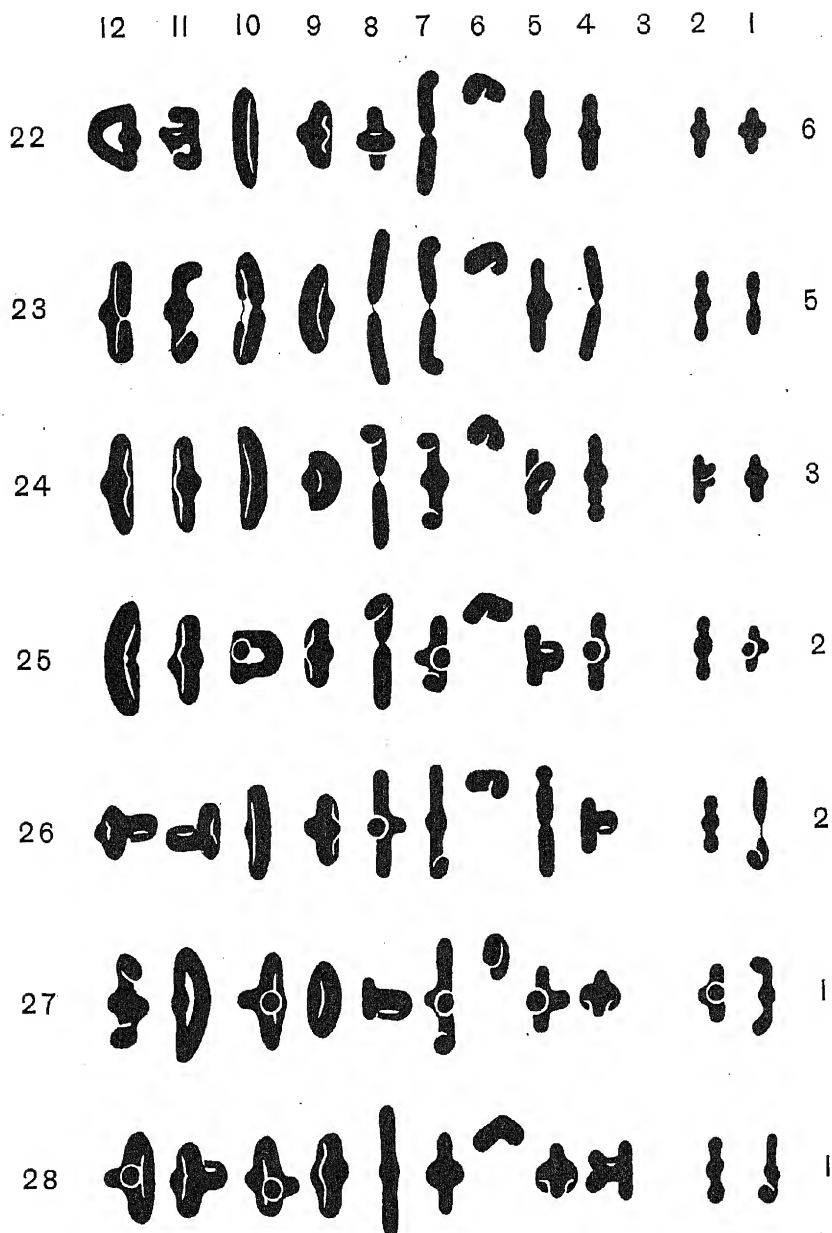
All complexes are from individuals of the Michigan group.



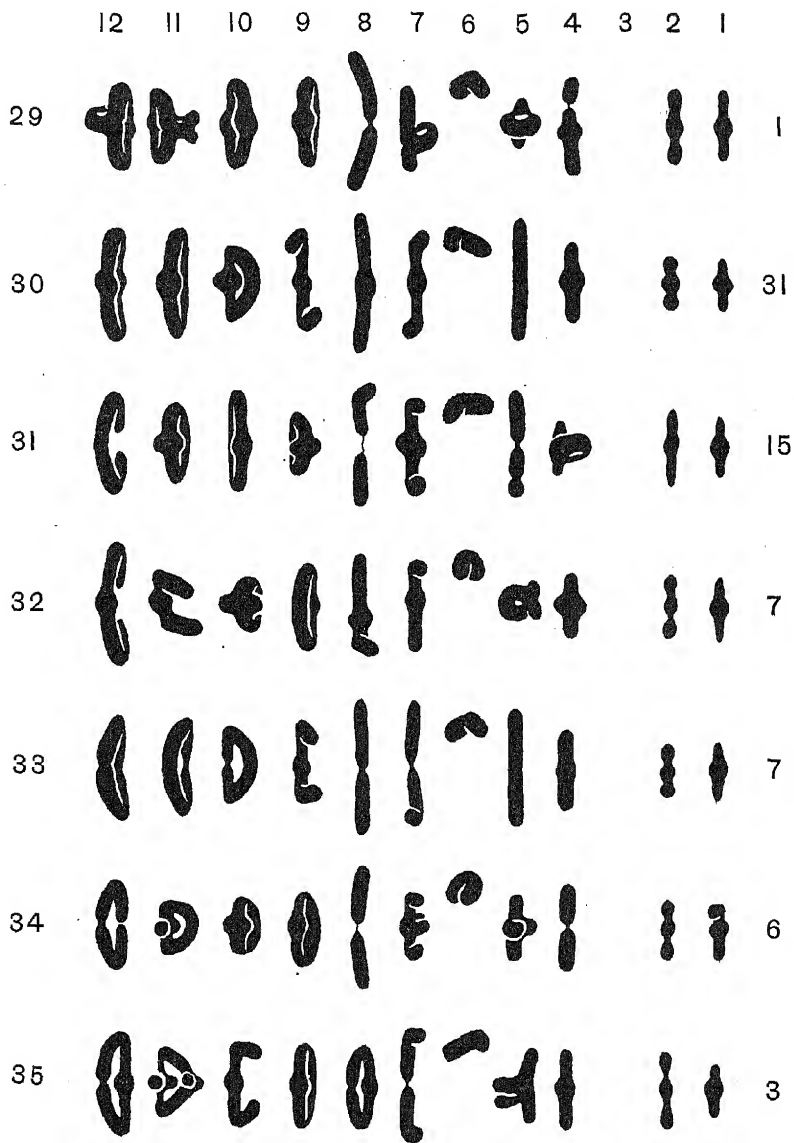
All complexes are from individuals of the Michigan group.



15 to 20 Complexes are from individuals of the Michigan group.
21 Complex is from an individual of the Greylock group.

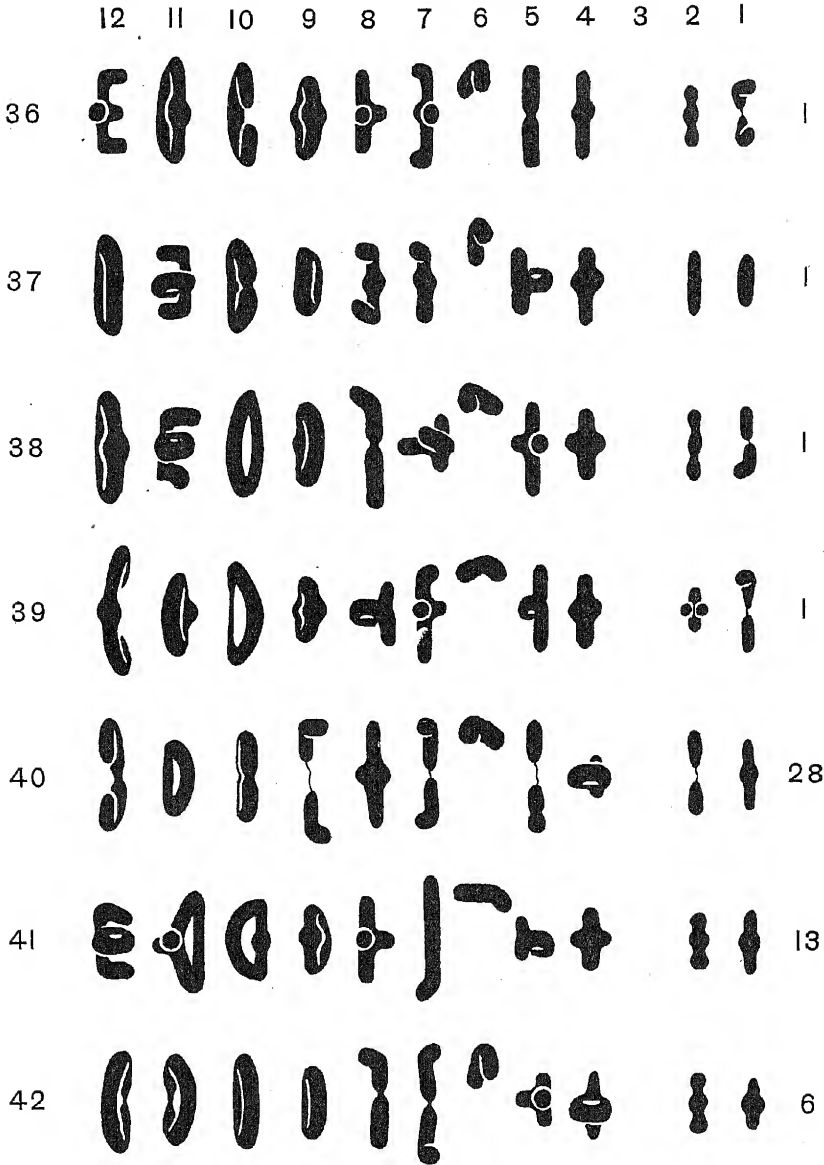


All complexes are from individuals of the Greylock group.

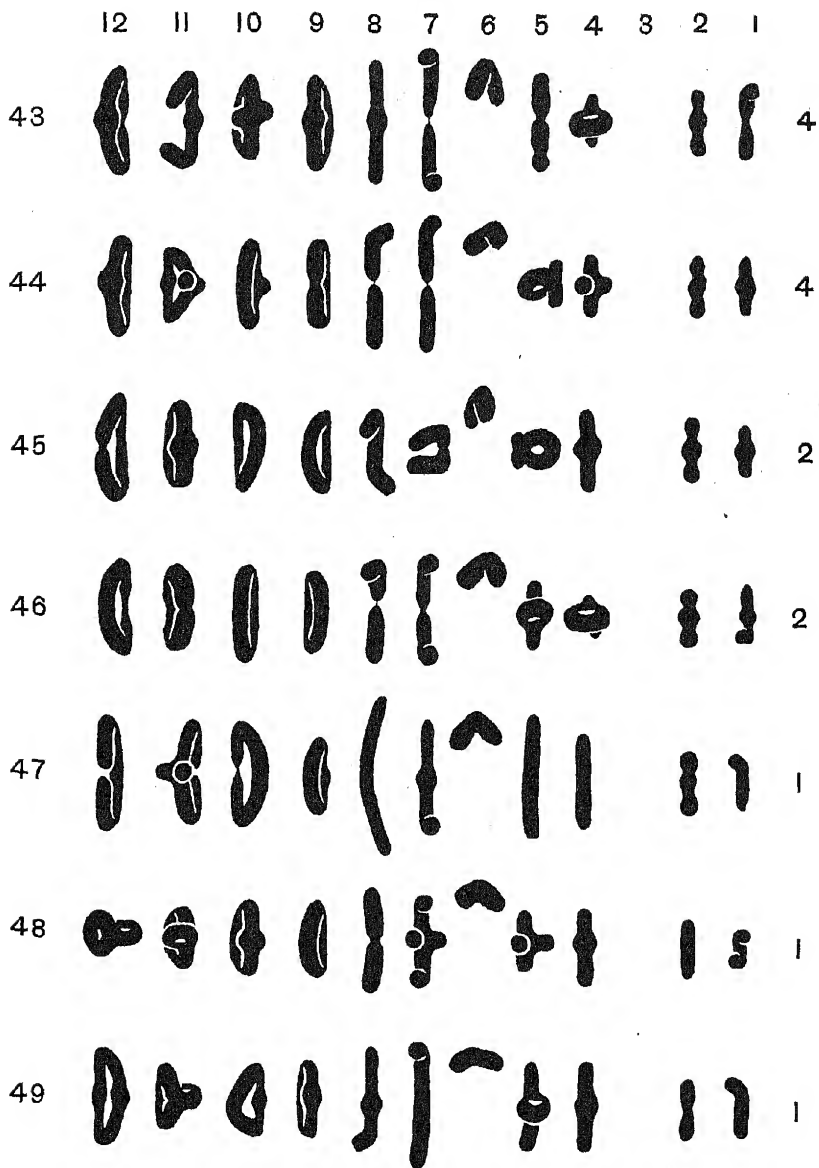


29 Complex is from an individual of the Greylock group.

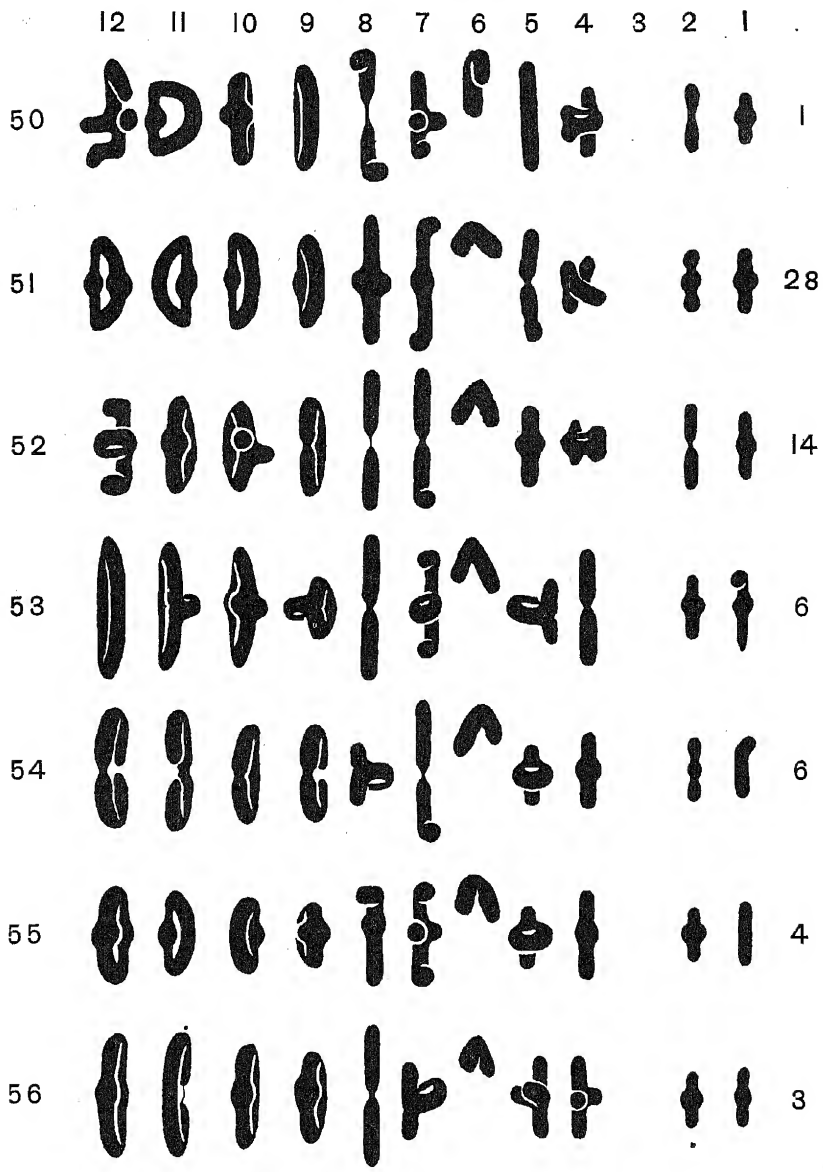
30 to 35 Complexes are from individuals of the Manchester group.



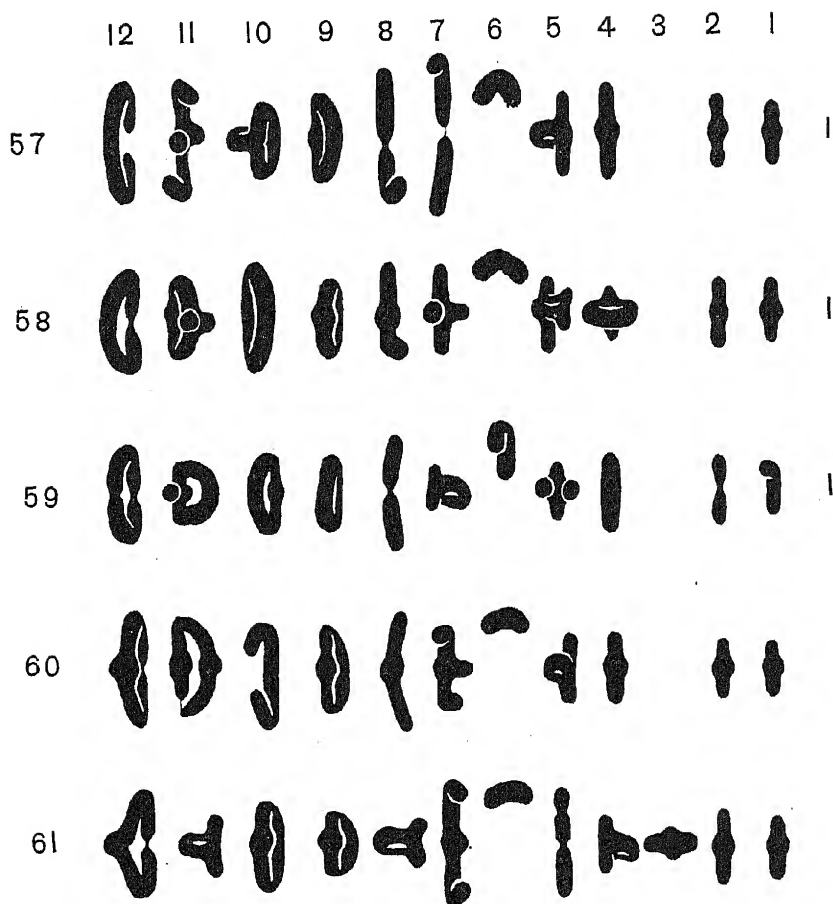
36 to 39 Complexes are from individuals of the Manchester group.
40 to 42 Complexes are from individuals of the Wachusett group.



All complexes are from individuals of the Wachusett group.



50 Complex is from an individual of the Wachusett group.
51 to 56 Complexes are from individuals of the Mount Desert Island group.



57 to 59 Complexes are from individuals of the Mount Desert Island group.
60 and 61 Complexes are from the same individual; in 60 the octad multiple, no. 11, is partially dissociated at one end. In 61 the multiple is completely dissociated and the component tetrads are shown in columns 11 and 3. These two figures are from Carothers ('21), plate 2, figures 13 and 14.

BINARY FISSION IN THE AMOEBOID AND FLAGELLATE PHASES OF TETRAMITUS ROSTRATUS (PROTOZOA)

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SIX HELIOTYPE PLATES (FIFTY-FIVE FIGURES)

AUTHORS' ABSTRACT

The vesicular nucleus of this amoeboid flagellate is similar in structure in both phases of its life-cycle. It has a fairly large caryosome surrounded by a pericaryosomal area in which there are small oxyphilic pericaryosomal granules on a fine reticulum. On the inner surface of the definite caryotheca is a layer of epithelial chromatic granules.

Nuclear division is similar in both amoeba and flagellate phases. During the prophase the nucleus enlarges, and the expanded caryosome becomes resolved into basophilic and oxyphilic components and assumes either an oblong, dumb-bell, or spindle shape. The pericaryosomal granules enlarge, shift about, and eventually become arranged in an equatorial band around the elongated caryosome. In the metaphase the equatorial plate of chromosomes appears after the inward migration of the pericaryosomal granules, accompanied by the formation of a definite intranuclear spindle, usually with polar masses, polar granules, and a centrodosome. After the poleward migration of the daughter plates of chromosomes in the anaphase, the telophase constriction of the nuclear membrane produces two daughter nuclei with a portion of the spindle remaining outside. The epithelial layer of granules remains in place on the nuclear membrane during the entire process of mitosis. Plasmotomy normally follows mitosis, but may be delayed, giving rise to multinucleate individuals. In the flagellate the blepharoplast usually divides simultaneously with, but independently of, the nucleus. There are many divergences in the details of mitosis, but these are thought to be variations of one type of division rather than examples of different processes.

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INTRODUCTION

In a previous paper (Bunting, '26) an extended account of the life-cycle of *Tetramitus rostratus* was presented. It is the purpose of the present paper to describe the details of binary fission, especially the nuclear divisions, in both the amoeboid and flagellate phases of this protozoon. A brief review of the life-cycle will afford a background for these division processes.

Brief review of the life-cycle

It will be recalled that the amoeboid phase transforms into the flagellate one, and vice versa. Pure-line cultures established by isolations were employed to determine the life-cycle. Living animals were frequently observed in the process of transformation, both in the isolated individuals of hanging-drop cultures and also in hanging-drops made from pure-line Petri-dish cultures.

A typical life-cycle may be outlined as follows: Cysts when planted in an appropriate medium excyst into amoebae which may undergo division several times, but some of them eventually transform into flagellates. In this latter phase division also takes place and the flagellate condition may continue for several days or sometimes for several weeks or even months, but finally the flagellates transform back to amoebae, which,

after a short time, with or without further multiplication, become encysted.

The flagellate phase is conical in shape and measures commonly from 14 to 18 μ in length and 7 to 10 μ in width. At the anterior end is located the blepharoplast, usually paired, from which arise four delicate flagella. A rhizostyle continues posteriorly from the blepharoplast and generally passes near to and beyond the nucleus. The vesicular nucleus, located anterior to the center of the body, is usually 3 to 5 μ in diameter. The individuals are commonly uninucleate, but binucleate and multinucleate forms are sometimes found. At one side of the flattened anterior end is a projecting beak or rostrum which is traversed by a cyto-stomal groove that extends posteriorly into the body. In some preparations the margins of the lips on either side of the groove contain definite fibrils. The contractile vacuole is also located in the anterior region.

The amoeba phase is of the limax type with usually one pseudopodium forming at a time. The vesicular nucleus is generally 5 to 6 μ in diameter. The contractile vacuole is commonly single, but may be augmented by smaller ones, and its position varies during locomotion.

The cysts are normally spherical, but occasionally are broadly ovoid. They are generally uninucleate, but binucleate cysts sometimes occur, and rarely multinucleate cysts are found. However, no stages of nuclear division have ever been found in the cysts. In the mature cyst the nucleus has a small caryosome and may be obscured by chromatic granules in the cytoplasm which are often very numerous. The cyst is bounded by a smooth delicate wall which dissolves during excystation. Living and fixed cysts show a range in diameter from 6 to 18 μ .

Materials and methods

Cultures. As recorded in the previous paper, the original organisms were obtained in cultures of the caecal contents of rats. During the study of the life-cycle a considerable

number of culture methods were employed. The principal media have been: Musgrave and Clegg's ('04) amoeba agar; Sellard's ('11) liquid medium; Sellard's formula with dextrose substituted for lactose; the latter Sellard's medium with 1.5 per cent agar added; ovomucoid medium; spinach extract, with and without dextrose, and spinach extract, with 1.5 per cent agar added. Frequently, the agar plates were flooded with a layer of Sellard's fluid before or after inoculation. Further details are given in the previous paper.

Fixing and staining. Three methods were used in the preparation of slides, all apparatus used being carefully sterilized. 1) A small amount was taken from the culture by a pipette and dropped on a cover-glass, which was immediately placed in a damp chamber, in which it remained for two or more hours until slight evaporation had occurred. The drop was then spread out thin and quickly plunged face upward into the fixing solution. 2) Covers were carefully floated on liquid media for varying periods, after which they were removed and placed face downward upon the fixative. 3) Covers were pressed down slightly upon solid media, allowed to remain for several hours, then removed by forceps and floated face downward on the fixative. The greatest number of flagellates were obtained by the first method. Adherence to the cover-glass was most satisfactory with material from 10 to 15 per cent ovomucoid cultures. In the cases of more fluid cultures, such as spinach extract, modified Sellard's fluid, etc., a drop of 15 per cent to 20 per cent ovomucoid was placed on the cover to which a drop of the above cultures was added, and then the cover treated as in method 1.

Several fixing agents have been employed. The best results have been obtained with warm Schaudinn's fluid to which five parts of acetic acid had been added. Among the other fluids used were chromacetic, Flemming's fluids, both weak and strong, Heller's, Bouin's, and Zenker's fluids. All these solutions were warmed before using.

Various staining methods were tried. The most dependable proved to be the iron-alum haematoxylin (longer)

method, followed by counterstaining with either alcoholic orange G or alcoholic eosin. The latter counterstain was usually the more satisfactory. Other stains used were Mayer's haemalum, with or without eosin as a counterstain; ferric-chloride haematoxylin; phosphotungstic-acid haematoxylin; Mann's methyl blue-eosin; safranin and orange G; safranin, gentian violet, and orange G (Flemming's triple stain), and Giemsa's stain (wet method).

OBSERVATIONS

Structure of the nucleus

As described in the previous paper, the 'resting' nucleus of both the amoeboid and flagellate phases is vesicular in structure with a fairly large caryosome. In the division stages the caryosome resolves itself into at least two components, a more intensely staining basophilic substance and a matrix which stains less readily with basic dyes. As a rule, in the resting condition the caryosome is smoothly rounded and homogeneous in appearance, which is considered normal. Occasionally, however, it may be irregular in profile or may show a differentiation into chromatic granules, strands, or a network, in a non-chromatic matrix, or it may be broken up into a few larger or many smaller rounded fragments. These more exceptional appearances are not regarded as normal. It has not been possible to identify a centriole or division center in the caryosome in these resting nuclei, though there have been appearances which suggested such a structure.

Outside the caryosome and within the caryotheca is an area that remains clear in prepared material, which will be referred to as the pericaryosomal area. It contains oxyphilic granules that usually stain with such dyes as eosin or orange G. We shall refer to these as the pericaryosomal granules. These granules, in most cases, appear to be accompanied by very fine threads, which may be either in the form of a reticulum, or of radiating fibers, or both. Sometimes the granules appear to be arranged in a single layer about midway between

the caryosome and the periphery of the nucleus, but at other times they are more irregularly distributed (figs. 1 and 2).

The nucleus is surrounded by a thin, but definite nuclear membrane, or caryotheca, to the inner surface of which is attached a layer of bead-like granules that stain intensely with the basic dyes. We shall call these the epithecal granules. From without inward, therefore, we have the following layers or zones: 1) caryotheca with its epithecal layer of chromatic granules; 2) pericaryosomal area containing the pericaryosomal granules and the accompanying network, and, 3) the caryosome with its several components.

The chromaticity of the nucleus, as determined by haematoxylin staining, varies with the phase of the life-cycle, the amoeboid phase showing the most basophilic material and the cyst stage the least. As reported in the previous paper, there is some indication that during transformation from amoeba to flagellate, the amount of basophilic material may be reduced by the extrusion of granules into the cytoplasm (Bunting, '26, p. 36). In the cyst the size of the caryosome gradually diminishes, while at the same time there is a gradual accumulation of chromatic granules in the cytoplasm. The epithecal layer is usually not so prominent in the flagellate and is still less so in the cyst. In fact, if not especially looked for, this layer might readily be missed in the cyst stage and sometimes in the flagellate.

In addition to these variations associated with the diverse phases of the life-cycle, there are also differences which appear to be dependent upon varying physiological (metabolic) conditions, probably associated with the different kinds of culture media and the progress of changes going on in them. There are also differences in appearance due to varied methods of technique.

Division of the amoeba

The nuclear division of the amoeboid and flagellate phases are essentially similar, but since we have had a larger amount of material containing the amoeboid phase, we have been able

to secure a more complete series of stages in this phase, hence this more complete account of the division of the amoeba is presented first.

There are many variations in the details of the process, and one series of drawings, figures 28 to 32, illustrates a more unusual course of events. In the description which follows it will be understood that all drawings are from material fixed in Schaudinn's and stained in iron-alum haematoxylin, unless otherwise noted.

Prophase. As is commonly the case in this class of organisms, preparation for division is accompanied by increase in size of the nucleus. This is well illustrated by the early prophases shown in figures 2 and 3 in comparison with the 'resting' nucleus of figure 1. This increase in size, which involves the entire nucleus, may be considered as the starting-point for the prophase stages.

Following the enlargement of the nucleus, various internal changes become evident, of which the most noticeable are in the caryosome. The enlarged caryosome becomes resolved into a more deeply staining component and a less deeply staining one, but the details of these changes are highly variable. As shown in figure 3, there may be a slight elongation of the caryosome accompanied by its resolution into a coarse chromatic network and a lightly staining matrix. This stage may be followed either by a further elongation into a broadly spindle-shaped body in which the network becomes finer with a longitudinal arrangement more pronounced (fig. 4); or a rectangular body with coarse reticulum still evident (fig. 6); or with the reticulum showing much finer meshes (figs. 5 and 8); or the caryosome may elongate and display a series of medially arranged vacuoles (fig. 7) instead of a reticulum. In figure 9 it will be seen that the lengthening of the caryosome has been accompanied by the assumption of a dumb-bell shape—a form frequently seen; but other shapes are almost as common.

Along with these changes occurring in the caryosome there are important ones taking place in the pericaryosomal area,

where the granules undergo various shiftings in position represented by condensation on one side (fig. 5) or lining up in rows on opposite sides (fig. 7); but eventually they become concentrated in an equatorial ring about the forming spindle (figs. 8 and 9).

Metaphase. From these prophases there arises a broadly spindle-shaped metaphase figure with numerous small chromosomes in an equatorial plate. It has been impossible to count the number of these small chromosomes. The equatorial-plate stage presents many variations which suggest that the prophase transformations may be equally various or that a great many changes in constitution of the spindle may take place while it remains in this stage. There is one feature common to all, however, which is, that with the formation of the equatorial plate, no more granules are to be seen in the pericaryosomal area. The condition illustrated by figure 10 may well have arisen by a further constriction of the caryosome and migration of the pericaryosomal granules into the spindle. The granules may then become arranged into an equatorial plate, as shown in figure 13. These two stages might well follow the prophase of figure 9 where the pericaryosomal granules are concentrated in an equatorial zone, but have not completed the inward migration.

When the chromosomes are arranged into an equatorial plate the ends of the dumb-bell-shaped caryosome become polar masses, with the centrodosome connecting them (fig. 13). This metaphase of figure 13 could have been derived from the prophase stage of figure 9 by a fading out of the 'handle bar' of the dumb-bell and a retention of chromaticity by the terminal knobs. In figure 11 there is shown a spindle-shaped condition of the central part of the nucleus with granules distributed along the spindle, but without an equatorial plate. This might well have developed from the stage of figure 4 by a migration into the forming spindle of the pericaryosomal granules, to be followed by the formation of an equatorial plate, as seen in figure 14. It will be noted that in the three figures just mentioned the polar masses are not conspicuous, but the centrodosome is definitely shown.

In figure 12 there is illustrated a metaphase stage comparable to that of figure 14, but the spindle is more pointed and there are no granules on it other than those of the metaphase plate. This figure is from a slide fixed with chrom-acetic, and the differences noted may be due to the variation in technique. The metaphase shown in figure 16 is from a slide fixed in Zenker's fluid. Here, again, technique might account for the differences, in part, but in this case the polar masses appear to have become broken up into granules.

Occasionally, the caryosome remains chromatic up to the metaphase (as illustrated by fig. 17), so that the entire spindle is deeply stained, though faintly reticulate, except a narrow clearer zone on either side of the metaphase plate. Such a condition may well have arisen from a prophase like that shown in figure 8, where the elongated caryosome has a similar structure. Intermediate conditions between the more chromatic and less chromatic spindles are illustrated by figures 15 and 18, and still others have been omitted from the plates.

Observations on living amoebae, as reported in the earlier paper (Bunting, '26), disclosed that, a short time before the cell body began to divide, the animals went through a 'gel' state. Some metaphases have been found on the slides in which there was little differential staining. It is possible that such faintly staining stages may represent these 'gel' conditions. On the other hand, it is possible that this 'gel' state may interfere with the changes going on in the spindle and thus account for some of the variations observed.

Anaphase. As is commonly the case, the anaphases have been less numerous than the other mitotic stages on the slides showing divisions. However, they might be expected to vary as much as the preceding metaphases, unless there are extensive adjustments during the metaphases. As a matter of fact, except for the stages on plate 4, we have not observed so many different conditions in the anaphases as we have in the prophases and metaphases; but this may be due, in part, to smaller total number of anaphases examined.

Figures 19 and 20 represent typical conditions in the anaphase. Figure 19 shows a much elongated nucleus with daughter plates of chromosomes well up toward the poles. The polar mass at the upper end appears to include a polar granule (centriole?), and the somewhat crescent-shaped polar mass of the lower end, with its irregular inner profile, presents a typical condition as found in many late anaphases and early telophases. Such a stage might logically follow the metaphase shown in figure 13, while the anaphase of figure 20 corresponds more closely with the metaphase of figure 14. The heavily stained polar masses of the anaphase illustrated in figure 18 suggest that this stage arose from a metaphase such as is depicted in figure 17, where there has been little change in the chromaticity of the caryosomal material while the spindle was forming.

Aside from the exceptional conditions figured on plate 4 and described beyond, the later anaphases do not seem to show so many variations as the earlier ones. During these later stages, the nucleus becomes more and more elongated until it may be several times as long as wide (figs. 20, 21, 22). Variations are to be seen in the daughter groups of chromosomes, which are sometimes well defined (figs. 19 and 20), even in an early telophase (fig. 23), while at other times they become more or less clumped and ill defined (fig. 21). The centrodesmose is traceable through the entire length of the spindle in these later anaphases (figs. 20 and 22), and in some cases the polar granules are distinctly evident (fig. 20).

Telophase. The stretched caryotheca of the elongated anaphase appears to constrict and then give way about the equator, and the free edges retract along the central remnants of the spindle toward the poles where they round up (figs. 22 to 24). There is thus left remaining outside the newly formed daughter nuclei a portion of the central part of the spindle (figs. 23 and 24).

In all cases the newly formed daughter nuclei contain a central area, which includes two masses or groups of granules that stain more intensely than the remainder of this area.

One of these masses, or groups of granules, represents the polar caps of the spindle and the other represents the daughter groups of chromosomes (figs. 24 to 27). It has been impossible to follow in detail the transformation of this central area into the resting nucleus with its rounded chromatic caryosome and its pericaryosomal zone containing the reticulum and the pericaryosomal granules.

One of the most significant features of the entire process of nuclear division is the persistence of the epithelial layer of chromatic granules. There are times during the anaphase when these granules are much less readily detected, but this is probably due to the stretching of the caryotheca resulting in the flattening out of these granules against this membrane (fig. 22). In a few cases, in late anaphases, the nuclear membranes at the poles exhibited an irregular profile ('amoeboid appearance'). In such cases this epithelial layer was less pronounced along this irregular polar area. However, as soon as the telophase nuclei round up (figs. 24 and 25), the epithelial layer can be distinctly seen throughout, and it is clear that it takes no part whatever in the formation of any constituent of the spindle.

Exceptional stages. On plate 4 is presented a series of drawings, illustrating conditions only occasionally met with. Yet some of them have been seen frequently enough to demonstrate that they may not be abnormal.

One unusual condition is the much elongated nucleus with the central area rather uniformly granular, as seen in figure 29. In such a stage the degree of elongation together with some tendency to constrict in the middle suggests imminent division of the nucleus. Yet in this case neither spindle fibers, chromosomal plates, polar masses, polar granules, nor centrodesmose are discernible. It is tempting to consider this as representing a distinct type of nuclear division. However, further study of the slides brought to light stages like those of figures 30 (Bouin's fixative) and 32 (Zenker's fixative), which resemble figure 29, except that definite nuclear plates are recognizable. It is therefore considered probable that

metaphase or anaphase plates are actually present in figures 29 and 31, but are masked by the numerous other granules present. In figure 28 (chromacetic) there is shown an early prophase in which the more chromatic component of the caryosome has become resolved into a large number of granules, while the pericaryosomal granules have not yet migrated inward. Such a prophase might readily develop into these unusual spindles of figures 29, 30, and 32. Another logical series could be arranged in the following order: prophases of figures 5 and 8, then the metaphase of figure 17, followed by the stages of figures 30 and 32, possibly to lead to the somewhat granular anaphase of figure 33. Figure 31, depicting an elongated nucleus with a central granular zone and large, compact polar masses, perhaps represents a stage intermediate between the metaphase of figure 17 and this completely granular spindle of figure 29, possibly with figures 30 and 32 as additional intermediates. Other stages with a less pronounced tendency to granulation may be seen in the metaphases of figures 11 and 14 and the anaphases of figures 21 and 22, with a more noticeable granulation in figure 33.

Figures 34 and 35 illustrate the multinuclearity that is sometimes found among the amoebae. In one extreme case a large amoeboid body contained twenty-one nuclei. These multinucleate conditions are regarded as abnormal and as representing a breaking down of the division mechanism, so that plasmotomy is prevented from occurring, while nuclear division proceeds without interruption.

Plasmotomy. Although it is the purpose of the present paper to lay chief emphasis upon the nuclear changes during division, it may be well to summarize here the observations on the divisions of the cell body that have already been published. The amoebae remain motile during almost the entire process of division, but they do not usually present the elongated limax shape that is commonly seen when no division is in progress. Some time before elongation for constriction occurs, the living amoebae enter into a 'gel' state which

may last for several minutes, but which is followed by a resumption of pseudopodial activity that soon becomes bipolarized. That is, as the animals elongate during the late anaphases and early telophases of nuclear division, pseudopodia appear at each end and probably aid in bringing about the separation of the cell body into two parts (figs. 23 to 26). As seen in figures 24 to 26, the middle region where constriction takes place is marked by more extensive vacuolization of the cytoplasm than elsewhere. This would doubtless add to the fragility of this region and aid in the separation process.

Division of the flagellate

As previously stated, the structure of the nucleus of this protozoon is essentially the same in both the flagellate and the amoeboid phases. The nucleus is, however, somewhat smaller in the flagellate, in harmony with the smaller cell body of this phase, and the epithelial layer of granules is less well developed. Although the structure of the flagellate is complicated by the presence of the cytostome and the blepharoplast with its outgrowths, the four flagella, the rhizostyle, and the oral fibrillae (fig. 36), the division of the nucleus parallels very closely that of the amoeba, as described in the section above, and, to a considerable extent, it is independent of the division of the blepharoplast.

Prophase. As indicated in figures 37, 38, and 39, the prophase changes begin with an expansion of the entire nucleus and a change in the organization of the enlarged caryosome, so that it becomes resolved into two components of different staining intensities. At the same time the pericaryosomal granules are seen to be more conspicuous and may be concentrated on one side of the caryosome (fig. 39), as was observed in the amoeba (fig. 5).

When the caryosome begins to elongate, it may present a variety of structural appearances similar to those found in the amoeba. Figure 40 shows a clear area in the middle of the caryosome—a condition frequently observed in the amoe-

bae—and it may well be comparable to the condition seen in figure 6 except that in the amoeba a reticulum has persisted that did not do so in the flagellate. The dumb-bell shape of the elongating caryosome, so frequently seen in the amoeba, is illustrated in figure 41, where it will also be noted that the pericaryosomal granules are becoming concentrated about the equator of the elongating nucleus. As the central part of the nucleus takes on the form of a short spindle, the pericaryosomal granules disappear from the pericaryosomal area and apparently become incorporated into the forming spindle (fig. 42).

Metaphase. When the equatorial plate appears (figs. 42 to 45) the middle part of the spindle is less chromatic, bringing into relief the more deeply staining polar masses and the centrodosome. The centrodosome in some cases (fig. 43) is seen to be attached toward the poles with definite polar granules. Figure 47 illustrates a short granular spindle in which the nuclear plate cannot be distinguished—a stage paralleling that of figure 11 for the amoeba.

Anaphase. Elongation of the metaphase stage (fig. 46) is accompanied by a rounding up of the cell body and is followed by the anaphases during which daughter groups of chromosomes arise and migrate toward the poles (fig. 48). Just as in the amoeba, the nucleus becomes much elongated before constriction occurs (figs. 48 and 49).

Telophase. Constriction is followed by the parting in the equatorial region of the caryotheca, which then retracts toward the poles and rounds up, leaving the central part of the spindle outside the forming daughter nuclei (figs. 50 and 51). During the anaphases and telophases the distinctness of the daughter plates of chromosomes varies considerably, as shown by their clumped condition in figure 48 as compared with their relatively well-defined condition in figures 49 and 50. In figures 49 and 50 it will also be noted that the centrodosome is clearly differentiated and that it extends the entire length of the elongated nucleus, ending in the polar masses which very probably contain the polar granules. With

the completion of the division of the nucleus, the daughter nuclei begin to reorganize themselves into typical nuclei, while the cell body constricts in the middle and finally separates into two (fig. 52) daughter flagellates.

Just as in the amoeba, the epithecal layer of chromatic granules appears to remain relatively unchanged throughout the entire process of nuclear division and, of course, the caryotheca likewise persists until severed by the telophase constriction. During the much elongated, stretched, and constricted condition of the nucleus, as seen in figures 49 and 50, it is not always possible to make out the epithecal granules in the middle region where the stretching is pronounced. The conditions shown in figure 51, where the caryotheca has obviously just broken at the equator and retracted to the poles, indicate a possible absence of these granules from that part of the membrane which had been excessively stretched. However, as soon as the daughter nuclei are completely rounded up, the epithecal layer is also seen to be complete (fig. 52).

Division of the blepharoplast. As a general rule, the blepharoplast divides simultaneously with the nucleus, but the time relations are subject to much variation. Often in the flagellates which show no division in the nucleus, one can see that there are two blepharoplasts lying close together, each giving rise to two flagella (fig. 36). In the process of division these two separate, but remain attached to each other by a deeply staining strand, the paradesmose (fig. 44). While there is some tendency for one or the other of the daughter blepharoplasts to be located near one pole of the dividing nucleus (fig. 44), a strictly polar position for both daughter blepharoplasts has never been seen. According to the prophases illustrated by figures 38 to 41, the daughter blepharoplasts do not begin to separate until the nucleus starts to elongate and a spindle is formed. On the other hand, an early stage in this separation of daughter blepharoplasts is represented in figure 37. Figures 42 and 43 illustrate metaphases where the daughter blepharoplasts have scarcely started to separate, while figure 44 shows a metaphase with blepharoplasts

widely divergent. Parallel stages in the separation of the blepharoplasts and division of the nucleus are indicated in figures 47 to 52.

Extreme examples of independence between the division processes of nucleus and blepharoplast are illustrated in figures 45 and 55. In figure 45 there are four blepharoplasts, while the single nucleus is still in the metaphase stage. In figure 55 division of the blepharoplasts has proceeded much further, since there are eight daughter blepharoplasts in an individual with only one nucleus in process of division. In the opposite direction one finds multinucleate animals with but a single blepharoplast, as indicated in figure 46. This drawing was taken from a slide on which a majority of the flagellates had two or three nuclei; however, some had only one, while others had four or more. These extreme conditions, of course, are exceptional and even might be considered abnormal, yet they very clearly demonstrate the independence of the divisions of the nucleus and blepharoplast.

Exceptional nuclear divisions. For a long time no flagellates with long granular spindles like those seen in the amoeba and illustrated in figures 29 to 32 were found. Eventually, however, such a spindle, as shown in figure 54, was discovered. In figure 53 there is a polar view of a spindle which probably is in about the stage represented by figure 54 or a little earlier. The somewhat irregular polar mass of deeply staining material is seen here and the rows of granules converging into it. This drawing is somewhat diagrammatic, since it was impossible to count with certainty the number of converging granular strands. Another unusual feature of this animal is the pseudopodium at the right-hand side of the blepharoplast, which indicates that division of the nucleus was taking place while the animal was undergoing transformation—probably from the flagellate to the amoeboid phase. If division and transformation were to be completed in a normal manner with loss of blepharoplast, it would be further evidence of the relative independence of the nucleus and blepharoplast in their divisions.

Plasmatomy. After division of the nucleus and of the kinetic system has been completed, two additional flagella grow out from each daughter blepharoplast, thus providing each with its full complement of four flagella. The daughter groups of organelles, including a contractile vacuole and a cytostome, become located at opposite ends of the elongated cell body, and a constriction gradually divides it into two individuals (fig. 52). It may be said that the plane of division here is essentially longitudinal, in harmony with the division process in flagellates in general.

DISCUSSION

In the preceding pages we have limited ourselves primarily to an account of our observations. In this section we shall offer some interpretations of our results and some comparisons with those of others. In this discussion we shall not attempt an extensive review of the literature, especially in view of the comprehensive treatment of division phenomena given by Belar in his recent book ('26).

Structure of the nucleus

We have described the nucleus of *Tetramitus* as 'vesicular,' because of the general use of this term for nuclei of this kind. This type of nuclear structure is the rule among the limax or Vahlkampfia group of amoebae—to which group the *Tetramitus amoeba* appears to belong—and is also very common among a large number of other Protozoa of all classes.

A limax amoeba is among the simplest of all protozoa in its cytosomal organization, yet the structure of the nucleus may be relatively complex. If we employ the criteria afforded by morphology, staining reactions, and behavior in division, we may identify the following components of the nucleus of *Tetramitus*: 1) the caryotheca or nuclear membrane; 2) the ground-substance or enchylema; 3) the epithelial layer of chromatic granules; 4) the oxyphilic granules, and, 5) the reticulum (linin?) on which they are distributed, both in the pericaryosomal area; and in the caryosome, 6) the basichro-

matic material, 7) the oxyphilic component (plastin?), and, 8) the substances of the polar granules and centrodesmose.

With this array of nuclear structures and substances before us, the three categories, chromatin, nucleolar substance, and caryolymph mentioned by Belar ('26), scarcely seem adequate. Calkins ('26) recognizes this greater complexity by listing membrane, enchylema, chromatin, linin, plastin, and kinetic elements as constituents of protozoan nuclei.

Theoretically, one might suppose that each different kind of material or structure would serve some special function. Although the full list of activities of the nucleus is still unknown in detail, yet it is usually recognized that in these simpler Protozoa, at least, the nuclear functions include the capacity to control or influence: 1) metabolism, by the elaboration of enzymes and other secretions; 2) kinetic activity, especially in nuclear division, and, 3) heredity.

The rôle of the nucleus in constructive metabolism is demonstrated by the cessation of anabolic processes upon the removal of the nucleus and there is a growing body of evidence that nuclear materials are passed out into the cytoplasm during vegetative activity (compare *Paramecium trichium*, Wenrich, '26).

In the case of *Tetramitus*, our interpretation must be largely indirect. The epithelial layer of chromatic granules may be thought of as having a metabolic function, since they take no part in the formation of either the chromosomes or the spindle during nuclear division. Their arrangement against the nuclear membrane places them in the most advantageous position for interaction with the cytoplasm. The layer is subject to change, being of different thicknesses under different cultural conditions and in different stages of the life-cycle. It seems to be reduced in the flagellate and also in the cyst, as compared with the amoeba.

Our observations indicate that the caryosome also contains materials with metabolic functions. In the transformation from amoeba to flagellate, the caryosome appears to become smaller—as does the entire nucleus—and there is evidence

(Bunting, '26) that granules of chromatic substance may leave the caryosome and pass out into the cytoplasm. Furthermore, when the amoeba becomes encysted the caryosome diminishes greatly in size, while at the same time chromatic granules accumulate in the cytoplasm. A somewhat similar condition in the cyst was described by Gläser ('12) for *Amoeba tachypodia* and by Dobell ('14) for *Amoeba lacertae* (*Vahlkampfia dobelli*, Hartmann, '14). If, as appears to be the case, basichromatic material is passed out of the caryosome (and also from the epithelial granules) to the cytoplasm in the cyst, such a process may be thought of as an adjustment reorganization whereby the supply of metabolic-controlling material in the nucleus becomes greatly reduced in harmony with the cessation of vegetative activities. The term 'trophochromatin' may well be applied to these nuclear components which appear to serve a metabolic function.

The intranuclear kinetic elements of *Tetramitus*, judged by the usual standards, are the polar granules, the centrosomes, and the achromatic spindle. It is doubtful if all these are composed of the same kinds of materials, but the exact rôle of each is undetermined at the present time. In the evolution of the more complex Protozoa, kinetic structures become more highly elaborated and transferred in part to the cytoplasm, where they occur as basal granules of flagella and of cilia, extranuclear centrioles, etc., with or without a residual connection with the nucleus (rhizoplast). Division centers often remain within the nuclei of Protozoa, while in the nuclei of Metazoa little or no trace of an intranuclear kinetic component is left. In the flagellate stage of *Tetramitus* we have extranuclear kinetic structures represented by the blepharoplast with its outgrowths, while there is retained within the nucleus the series of spindle elements referred to above. With the absence of a rhizoplast, the extranuclear and intranuclear kinetic elements seem to be independent of each other.

The hereditary component of the nucleus is customarily identified as the substance of the chromosomes. Our observations lead us to the conclusion that the chromosomes are derived primarily, and perhaps wholly, from the oxyphilic pericaryosomal granules—a point discussed more at length further on.

In the previous paper (Bunting, '26) evidence was presented that during multiple fission some flagellates were formed without regular nuclei, but containing chromatic granules which could readily be interpreted as chromidia. Since we were not able to isolate such individuals and determine their subsequent history, we are unable to use these observations effectively either for or against the hypothesis of the chromidial origin of nuclei, although the evidence, so far as it was available, seemed to favor this hypothesis.

Nuclear division

Types of mitosis. Various classifications of mitoses in the Protozoa have been proposed. Chatton ('10) distinguished three types, as follows: 1) *promitosis*, in which the caryosome is formed mainly of plastin impregnated with chromatin and includes the centrioles; the chromosomes are derived from material outside the caryosome and the nuclear membrane persists; 2) *mesomitosis*, in which the division center (centriole) is outside the caryosome, from which the plastin component has entirely or largely disappeared, and the nuclear membrane persists; 3) *metamitosis*, in which the division center (centriole) is outside the nucleus and the process of mitosis is comparable to that in the Metazoa. Alexeieff ('13) has proposed a more elaborate scheme of classification, while less complex groupings have been suggested by Belar ('26) and by Wenyon ('26). We are disposed to agree with Calkins ('26) that an adequate, fully developed classification of the division processes of Protozoa is impossible at the present time. The less elaborate grouping of Chatton is, perhaps, the most useful until our knowledge is more complete. The nuclear division of *Tetramitus* fits fairly well into Chatton's *promitosis* group.

Origin of the chromosomes. There have been differences of opinion as to how far the term chromosome may properly be applied to nuclear structures in the Protozoa. Calkins ('26) applies the term to "those compact intranuclear aggregates of chromomeres which divide as unit structures and which are resolved into chromomeres after such division" (p. 115). Since this definition requires an additional explanation of the word 'chromomere,' we suggest the following: a chromosome is one of a group of compact bodies which develop out of nuclear materials during mitosis and become separated, each into two (usually) equal parts on the mitotic spindle. It is somewhat easier to identify the chromosomes than it is to identify the substance 'chromatin.' In the Metazoa the older term, linin, has been replaced by the term oxychromatin, and it is considered that oxychromatin and basichromatin are different expressions of the same basic substance, chromatin (Wilson, '25). Such a simple solution may not be adequate for the Protozoa where 'trophochromatin,' 'idiochromatin,' and 'kinetochromatin,' all of a basophilic nature, have been identified, in addition to oxyphilic 'linin' and 'plastin.'

In *Tetramitus*, as previously stated, the chromosomes of the spindle appear to arise from the oxyphilic granules of the pericaryosomal area. Are they, then, composed of oxychromatin? If so, what of the network on which they rest? Is it also oxychromatin? One interpretation of the prophases, such as shown in figure 9 where the caryosome becomes dumb-bell shaped and the pericaryosomal material concentrates around the equator, would be that the material of this network gives rise to the achromatic spindle. Other stages, however, for example, figures 4, 10, and 11, could be interpreted as showing that the spindle is derived from the caryosome. Unfortunately, our observations do not lead to a definite decision of this question.

As the chromosomes take their place on the equatorial plate they become more chromatic. This could be interpreted as evidence that basichromatin from the caryosome went into

their composition. It seems to us more probable that the increased chromaticity is due to physical condensation which increases their capacity to retain the haematoxylin stain. Belar ('26), in his figure 253, presents an extended series of sketches illustrating the structure and division of nuclei for the main groups of Protozoa and for some of the lower plants. According to his scheme for Vahlkampfia, the chromosomes are derived entirely from the chromatin of the pericaryosomal area. A similar interpretation has been given for this type of amoeba by many investigators, for example, by Wasielewski and Kuhn ('14) for different species of Vahlkampfia, by Jollos ('17) for several species of the same genus, and by Ivanic ('26) for Hartmannella testudinis, while others describe the derivation of the chromosomes from the caryosome, as, for example, by Vahlkampff ('05) for 'Amoeba limax,' by Nägler ('09) for several amoebae of the limax type, by Whitmore ('11) for culture amoebae, by Gläser ('12) for Amoeba lamellipodia, and by Dobell ('14) for his Amoeba lacertae (Vahlkampfia dobelli, Hartmann, '14), A. glebae, and A. fluvialis. Again, some authors believe the chromosomes to be derived from the layer of granules attached to the nuclear membrane (Gläser, '12, for Amoeba tachypodia), while still others believe that the chromosomes are derived in part from the caryosome and in part from the 'peripheral' chromatin (Aragao, '09, for Amoeba diplomitotica; Wilson, '16, for Nagleria gruberi). We are inclined to believe that future studies will reveal more and more cases in which the chromosomes arise from material outside the caryosome.

Origin of the spindle elements. Aside from the chromosomes, the mitotic spindle of Tetramitus includes, 1) the achromatic spindle fibers; 2) the chromatic polar masses or polar caps; 3) the polar granules (centrioles?), and, 4) the centrodosome. There is no question but that all of these elements are derived from the caryosome except the spindle fibers. As suggested on page 57, there is some evidence that these fibers may come from the reticulum of the pericaryosomal area, but other evidence indicates an origin from

the caryosome. A definite decision has not been reached. Belar's ('26) schematic figure of *Vahlkampfia* shows the spindle derived from the caryosome, and this origin has been described by most authors who have studied this type of amoeba. Ivanic ('24, '26), however, believes that two spindles are formed in *Amoeba verrucosa*, *Hartmannella testudinis*, etc., one from the caryosome and one from the 'Aussenkern,' and that these two fuse together. Gläser's figures for *A. verrucosa* suggest a similar process, although he did not so interpret them. It seems that additional attention needs to be given to this phase of mitosis in order to determine all the facts.

It has already been suggested that much of the basichromatin of the caryosome has a metabolic function and therefore constitutes 'trophochromatin.' This chromatic material appears to divide amitotically, but into approximately equal parts during the prophases of division (figs. 9 and 10) to become the polar masses or polar caps (figs. 13, 15, etc.). These polar masses are variable in size and structure, and it is difficult to determine whether or not all the basophilic material of the caryosome goes into them. The granules which are irregularly distributed in the spindle, either metaphases (figs. 11, 13, 14) or anaphases (figs. 21, 22, 33, 34), are probably of the same origin, but do not become concentrated in the polar masses. The 'Mittelstück' or 'Zwischenkörper' seen in the anaphases of *limax amoebae*, as figured by Vahlkampf ('05), Gläser ('12), and others, also indicates that part of the basophilic material of the caryosome is to be found in the middle part of the spindle as well as in the polar masses.

The presence of polar granules with a centrodosome between them is clearly shown in our material, and we have many drawings of such stages which have not been reproduced here. The function of these structures is not clearly apparent. In some of the earlier metaphases (fig. 43) the polar granules are some distance from the ends of the forming spindle and the centrodosome is straight. In later

stages, as well as in some early stages, the polar granules are at the poles of the spindle and the centrodesmose is at the periphery of the spindle. These differences suggest that, in the earlier stages, the caryosome may elongate faster than the centrodesmose, while in later stages the reverse may be true. The more peripheral position of the centrodesmose may thus result from a displacement due to its rapid elongation. A still more marked elongation would tend to throw the centrodesmose out toward the nuclear membrane in the position described for the 'intradesmose' by Kofoed and Swezy ('21). In view of the interpretation offered here, it is doubtful if there is any real distinction between the centrodesmose of other authors and the intradesmose of Kofoed and Swezy.

There has been much discussion in the literature as to the existence or non-existence of an intranuclear centriole in the division of these vesicular nuclei. Belar ('26) believes that the term centriole is somewhat inappropriate for these polar granules, and Wenyon ('26) refers to them as intranuclear structures 'supposed to function as a centrosome' (p. 90). Gläser ('12), Dobell ('14), Jameson ('14), and others believe that intranuclear centrioles are of rare occurrence; but, on the other hand, such polar granules have been described and figured by so many authors that their wide occurrence cannot be denied. We do not always find these bodies in our preparations, but this failure is believed to be due to the technique or to a masking by other elements of the spindle. We have not, however, found complete evidence of their persistence in the resting nucleus. With Belar ('26) we may raise the question as to whether the separation of these polar granules may not be more passive than active, and the centrodesmose the more active agent in the elongation of the spindle rather than the 'centrioles.' The suggestion of Wasielewski and Kuhn ('14) that the entire caryosome may function as the division apparatus in this type of nucleus should also be carefully considered.

Persistence of caryotheca and epithecal granules. Except for the parting at the equator in the telophase, we have found

that the caryotheca persists throughout mitosis, as has been reported by many investigators for this type of mitosis. However, Nägler ('09) and Wasielewski and Kuhn ('14) report that a true nuclear membrane does not exist for the series of limax amoebae studied by them; and Dobell ('14) describes the nuclear membrane as disappearing during the division of *Amoeba glebae* and *A. fluvialis*. It is doubtful if any nucleus in these animals lacks a membrane, and it is possible that, in Dobell's species, attenuation during the anaphase makes the membrane difficult to observe rather than that it disappears altogether. On the other hand, in the metamitotic type of mitosis with extranuclear division centers, the nuclear membrane undoubtedly does break down as it does in the Metazoa.

We have not found any account in the literature of the persistence of the epithelial layer of granules as we have observed it in *Tetramitus*. It should be emphasized again that the thickness of this layer varies and that in some cultures of the flagellates it might easily be overlooked if no special effort were made to observe it. During later anaphases, also, when the nuclear membrane is much attenuated, the epithelial layer is correspondingly thin and easily missed. Careful study of our preparations, however, has assured us that this layer does persist through the entire life-cycle and throughout mitosis, except when subdivided into two parts by the constriction of the nuclear membrane in the telophases. It is interesting to note that, in his illustrations of the division stages of *Amoeba fluvialis*, Dobell ('14) shows the persistence of the nuclear membrane with a layer of granules attached to the inner surface up to an early anaphase (his fig. 68). In the late anaphase (his fig. 71), Dobell says that the nuclear membrane disappears to be reformed in the daughter nuclei. It is possible that, due to their attenuation, Dobell overlooked both the membrane and its attached granules in these anaphases. A similar interpretation might apply to the division figures of *Scytomonas pusilla*, as illustrated by Schüssler ('17) where the nuclear

membrane is represented as thick and chromatic in most of the division stages shown in his figures 1 to 14. Alexeieff ('14) describes briefly the division of the flagellate *Tetramitus rostratus*, and although he does not show an epithelial layer of granules, he does picture an unusually thick nuclear membrane which persists throughout the process of nuclear division, so that it is probable that he was really representing the epithelial layer. In Aragao's ('16) account of *Copromastix prowazeki*, which closely resembles *Tetramitus rostratus*, the epithelial layer is shown in the resting nucleus, but not in the division stages. Yakimoff's ('23) *Copromastix aragaoi* resembles *Tetramitus rostratus* still more closely, but he did not describe division. We are inclined to believe that other cases of the persistence of the epithelial layer of granules will be found if careful search is made for them.

Divergent types of mitosis. In the descriptive part of this paper we have called attention to wide variations in the appearances of some stages of division, especially the prophases and metaphases, and particular emphasis was placed upon the peculiar spindles illustrated by figures 29 to 32 and figure 54. These divergences call for interpretation. Doflein ('18) suggested that varying colloidal conditions of the nucleus influenced the process of division. Attention has already been called to the fact that, in living amoebae, dividing animals appeared to pass through a 'gel' state for a longer or a shorter time. It is reasonable to suppose that this state would affect the nucleus as well as the cytoplasm and that differences in colloidal consistency might readily influence the reactions of the nuclear components to the fixing and staining agents.

Besides differences due to these diverse physical and chemical factors, there appear to be pronounced variations in appearances associated with the varying rates of progress of the different processes involved in mitosis. If we consider, for example, the series of stages during the transformation of the densely chromatic caryosome into an elongated spindle-shaped body, the chromaticity of which may be greatly

diminished, and then the changes through which the pericaryosomal material passes in transforming into the equatorial plate that separates into two daughter plates, and assume that these two series of events proceed at different rates of speed in different cells, then it may be appreciated that varied pictures of the entire process would result. There are also differences in the details of the changes through which the nuclear materials pass. The varying chromaticity of the metaphases illustrated by figures 12, 13, 15, and 17, for example, may reflect differences in the physical organization and distribution of the chromatic part of the caryosomes as well as different reactions to the technique.

The granular spindles shown in figures 29 and 31 do not reveal any plates of chromosomes, but the similar spindles of figures 30 and 31 do show nuclear plates, hence it is believed that chromosomal plates are also present in the first two figures mentioned, but are completely masked by the granules with which the entire spindle is crowded. Figure 31 seems to be an intermediate condition between a more solid spindle, as shown in figure 17, and the completely granular spindles of figures 29, 30, and 32, and it is possible that the completely granular spindles arose from the more uniformly dense ones by a change in physical state to the form of granules. On the other hand, the granular condition of figure 28 indicates that this change to granules may take place in the prophase. Taking into consideration all facts before us, it seems more logical to interpret these various divergences in appearance as variations of one type of mitosis, rather than as representatives of two or more distinct types.

Variations in the process of nuclear division in different species of amoebae and flagellates have been recorded by a number of investigators. Aragao ('09) reported two types of mitosis in *Amoeba diplomitotica*. Some of his figures resemble ours rather closely, and it is possible that he was dealing with similar variations rather than with distinct types of divisions. Whitmore ('11) mentions two varieties of nuclear division in his second type of *limax amoeba*, but

they are not described in detail. Zulueta ('17) describes two different mitotic processes for *Wasielewskia gruberi*. Many of his figures resemble those of Aragao and our own, and it is possible that in his animal, also, a more complete series of stages might reveal intermediates indicating modifications of a single type of division rather than two distinct kinds. Doflein ('18) reports one type of mitosis in *Polytoma agilis*, in which the caryosome disappears and another type in which it persists and divides. He believes these differences can be explained by variations in the colloidal condition of the caryosome—in its more fluid state, it disappears; in a more viscid state, it persists and takes the stain. This suggestion is a valuable one and, along with the others that we have mentioned, fits the interpretation of these differences in terms of variations in one type of mitosis.

Extranuclear kinetic elements

The extranuclear kinetic apparatus of the *Tetramitus* flagellate consists of the blepharoplast and the several fibers attached to it, namely, the four flagella, the rhizostyle, and the cytostomal fibrils. So far as our evidence goes, this entire apparatus disappears when a flagellate transforms into an amoeba and forms again when the reverse transformation takes place. Although we have some evidence that granules are given off from the nucleus of the amoeba (Bunting, '26, text figs. 12 and 13), we have not been able to determine whether such granules become blepharoplasts, or chromidia, or are cast out of the cell entirely, or are digested. The origin of the extranuclear kinetic system thus remains a problem yet to be solved. The intranuclear origin of the blepharoplast as described by Wilson ('16), Jameson ('14), and others has therefore not been confirmed by this study, and Wenyon ('26) also fails to confirm this origin in the transformation of amoeba to flagellate in *Dimastigamoeba gruberi*, which he supposes to be identical with the subject of Wilson's studies.

The rhizostyle is sometimes definitely chromatic and at other times less deeply stained and difficult to observe. It passes into the cytoplasm beyond the nucleus and therefore is to be distinguished from a rhizoplast, for which we have no satisfactory evidence in this animal. The function of this rhizostyle is problematical. Besides serving as an anchorage for the blepharoplast, it may represent an evolutionary stage in the development of an axostyle, a parabasal body, or a chromatic basal rod.

The cytostomal fibrils in the lips on either side of the cytostomal groove also vary in their staining reactions, more commonly being non-chromatic. In some cases there appear to be several fibrils in each lip, instead of the usual one. This has been observed primarily in the cultures where many unusual phenomena were occurring, such as multiple fission, cell fusion, formation of 'giants' and 'dwarfs,' etc., and probably represents an abnormal multiplication of these elements comparable to the multiple blepharoplasts seen in figure 55. The cytostomal fibers apparently possess some powers of movement in correlation with the capacity of this part of the body to change its shape to some extent. During transformation from flagellate to amoeba, a thin membrane has been seen projecting and undulating like a fin, as observed in the living animal (Bunting, '26). It is possible that such fin-like structures are activated by the cytostomal fibrils. If they are capable of undulatory movements, they may be homologous with similar fibrils occurring in undulating membranes.

In the division of the kinetic apparatus one usually finds the blepharoplast early separated into two components, each with two flagella attached. As these two daughter blepharoplasts separate, a deeply staining strand of material is seen connecting them, like the paradesmose of trichomonad flagellates (Kofoid and Swezy, '15). When the animals elongate preparatory to constriction, this paradesmose breaks into two, and there is a strong temptation to think of the two parts as persisting as the rhizostyles of the daughter animals. If so, then the rhizostyle of the parent must be resorbed. We have

not been able to follow the history of the cytostomal fibers during division.

We have already emphasized (p. 52) the independence of the blepharoplast and nucleus in their division processes, each apparently having its own division mechanism. A similar independence is illustrated by Doflein ('18) for *Polytoma agilis*. This independence is in striking contrast to the division process in trichomonad flagellates where the blepharoplast functions as a division center for the nucleus (Wenrich, '21).

SUMMARY

1. *Tetramitus rostratus* was originally obtained as an amoebflagellate in cultures of the contents of the caecum of rats. Many culture media have been tested and many methods of technique have been employed in making the fixed and stained slides. In a typical life-cycle of *Tetramitus*, cysts give rise to amoebae of the limax or Vahlkampfia type which, after dividing a number of times, may transform into a flagellate with conical shape, four flagella, and cytostomal beak or rostrum. After a few days (sometimes weeks or months), during which division takes place, the flagellates transform back to amoebae, which sooner or later encyst (Bunting, '26).

2. The 'resting' nucleus is similar in structure in the two phases of the life-cycle, being 'vesicular' with a rather large caryosome which is surrounded by a relatively clear area, the pericaryosomal area, in which there are oxyphilic granules on a fine reticulum; and outside this is the caryotheca, on the inner surface of which is a layer of chromatic epithelial granules. Using the criteria of structure, staining reaction, and behavior in division, one may recognize the following nuclear components: caryotheca, enchylema, epithelial granules, pericaryosomal granules, pericaryosomal reticulum, and the caryosomal constituents, consisting of the basophilic part, the oxyphilic part, the polar granules, and the centrodesmose. It is believed that the substance of the epithelial granules and the basophilic part of the caryosome may be 'trophochromatin,' since they vary quantitatively and are not

divided qualitatively during division. Generative chromatin may be identified with the chromosomes which are derived from the pericaryosomal granules. The intranuclear kinetic functions are presumably associated with the achromatic spindle, the polar granules, and the centrodesmose.

3. It was not determined whether the achromatic spindle fibers were derived from the pericaryosomal reticulum or from the oxyphilic component of the caryosome. The behavior of the centrodesmose suggests that it is the more active agent and the polar granules the more passive agent in the elongation of the spindle. It is possible that, as suggested by Wasielewski and Kuhn ('14), the entire caryosome functions as a division apparatus for the nucleus.

4. In the amoeba the division of the nucleus begins with a prophase enlargement of the entire nucleus. The expanded caryosome becomes resolved into a basophilic component, which assumes various forms, and an oxyphilic matrix. As it elongates the caryosome may be spindle-shaped, oblong, or dumb-bell-shaped. The pericaryosomal granules undergo shiftings of position, massing at one side, or lining up in rows on opposite sides, and finally collecting in a band about the equator of the elongated caryosome. The central portion of the nucleus then assumes a definite spindle form. As the pericaryosomal granules complete their migration inward, an equatorial plate of chromosomes appears. The metaphase stage is highly variable in appearance, showing differences in chromaticity of the entire spindle, differences in size and organization of the polar masses, and the differentiation or not of polar granules and centrodesmose. In some instances the elongated spindles are so full of chromatic granules that all other spindle elements are obscured. Although the anaphases are less variable, there is some diversity among them in harmony with the differences in the metaphase. Daughter plates of chromosomes migrate toward the poles where they approximate, but do not reach, the polar masses. Polar granules and centrodesmose are often well differentiated. During the telophase the nuclear membrane constricts and rounds

up to form the daughter nuclei, while the central portion of the spindle remains in the cytoplasm. During the entire process of division the epithelial granules remain in place on the caryotheca and take no part in the formation of either the chromosomes or the spindle. The caryotheca persists, except when severed by the telophase constriction. With the completion of nuclear division, plasmotomy normally follows by elongation of the cell, the formation of pseudopodia at each end and constriction in the middle. In the constricting region the protoplasm becomes more vacuolated than elsewhere. Details in the reorganization of daughter nuclei were not followed. In some cases nuclear division is not succeeded at once by plasmotomy, and thus multinucleate amoebae arise. In the flagellate phase nuclear division parallels that in the amoeba in all details.

5. The wide divergences in the various mitotic pictures, especially in the metaphase and anaphase, are thought to be due in part to differences in the colloidal state of the nuclear materials (Doflein, '18) and in part to lack of synchronism in the several series of changes involved in mitosis, as well as to other differences in the physical constitution of the basophilic part of the caryosome, which may be in the form of granules, a reticulum, or non-staining material. This interpretation of variability is preferred to that of diverse types of mitosis.

6. In the flagellate the blepharoplast is the center of the extranuclear kinetic apparatus which includes the four flagella, the rhizostyle, and the cytostomal fibrils. The blepharoplast normally divides simultaneously with the nucleus, but the time relations are subject to much variation. Although the daughter blepharoplasts are connected with each other by a deeply staining strand, the paradesmose, they do not become stationed at the poles of the dividing nucleus, hence cannot function as extranuclear division centers. The nucleus may divide independently of the blepharoplast, and vice versa, giving rise to multinucleate flagellates with but one blepharoplast and to others with from four to eight blepharo-

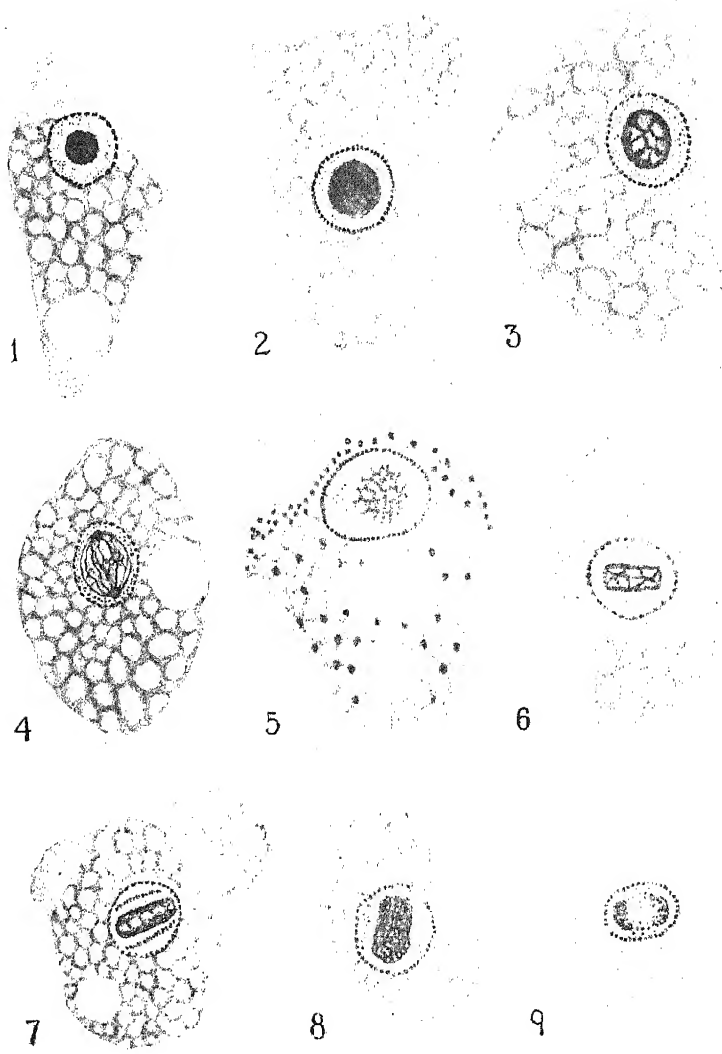
plasts and but a single nucleus. It is thought probable that the paradesmose, when parted in the telophase, may persist as the rhizostyles of the daughter flagellates. The fate of the parent rhizostyle and of the cytostomal fibrils was not followed. The origin of the blepharoplast remains an unsolved problem. It seems to disappear when a flagellate transforms into an amoeba and to be formed anew when the reverse transformation takes place. In some preparations there was evidence that chromatic granules are passed out of the caryosome into the cytoplasm, but the fate of such granules could not be determined.

7. The division of the nucleus of *Tetramitus* may be considered as an example of promitosis, as defined by Chatton ('10). Any attempt at an extended and complete classification of the nuclear divisions of the Protozoa is regarded as premature at the present time.

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DESCRIPTION OF FIGURES

The figures have been outlined under a camera lucida at a magnification of 4000 diameters and reduced $\frac{1}{3}$ in printing. Unless otherwise stated, they are all taken from material fixed in Schaudinn's fluid and stained with iron-alum haematoxylin. Plates 1 to 4 show figures of the amoeboid phase; plates 5 and 6, of the flagellate phase.

PLATE 1

EXPLANATION OF FIGURES

- 1 Vegetative amoeba with nucleus in 'resting' state.
- Figures 2 to 9 Prophases.
- 2 Early prophase; nucleus expanded.
- 3 Early prophase; enlarged caryosome differentiated into basophilic reticulum and oxyphilic matrix.
- 4 Spindle shape of caryosome; reticulum fibers mostly longitudinal.
- 5 Caryosome reticulate; pericaryosomal granules massed on one side.
- 6 Reticulate caryosome oblong; pericaryosomal granules collected toward opposite sides.
- 7 Elongated caryosome vacuolated through the center; pericaryosomal granules in rows on opposite sides.
- 8 Oblong caryosome finely reticulate; pericaryosomal granules concentrating about the equator.
- 9 Caryosome dumb-bell-shaped; pericaryosomal granules migrating inward.

PLATE 2

EXPLANATION OF FIGURES

Figures 10 and 11 Late prophases or early metaphases.

10 Caryosome much constricted; granules distributed along entire length of spindle.

11 Granular spindle; chromosomal plate not discernible; centrodosome visible.

Figures 12 to 17 Metaphases with definite plates of chromosomes.

12 Faintly staining spindle; centrodosome stretched between polar granules. (Chromacetic.)

13 Polar masses compact; centrodosome showing; some granules in the spindle.

14 Similar to figure 13, but polar masses much reduced.

15 Larger polar masses still reticulate like caryosome of figure 8. Centrodosome not visible.

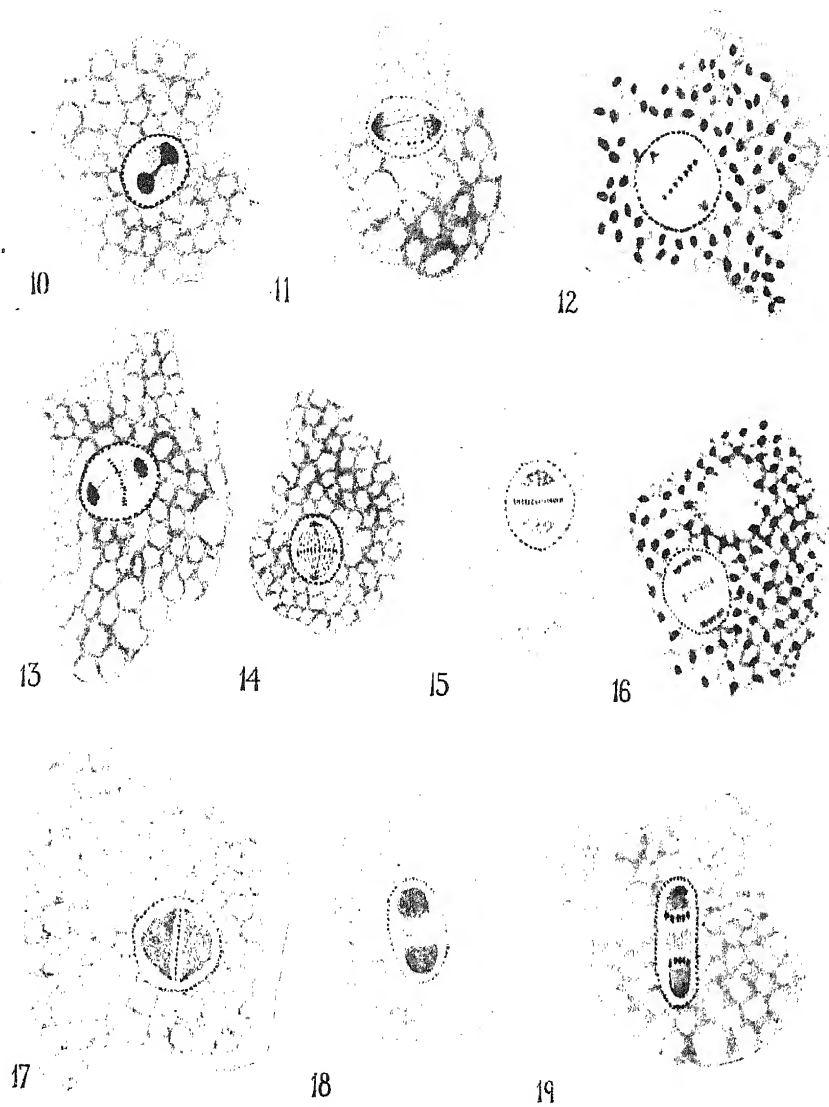
16 Polar masses broken up into granules. (Zenker's fixative.)

17 Spindle heavily stained, masking all spindle elements except equatorial plate.

Figures 18 and 19 Anaphases.

18 Early anaphase with large, heavily stained polar masses.

19 Later anaphase. Typical condition. Chromosomes well separated.



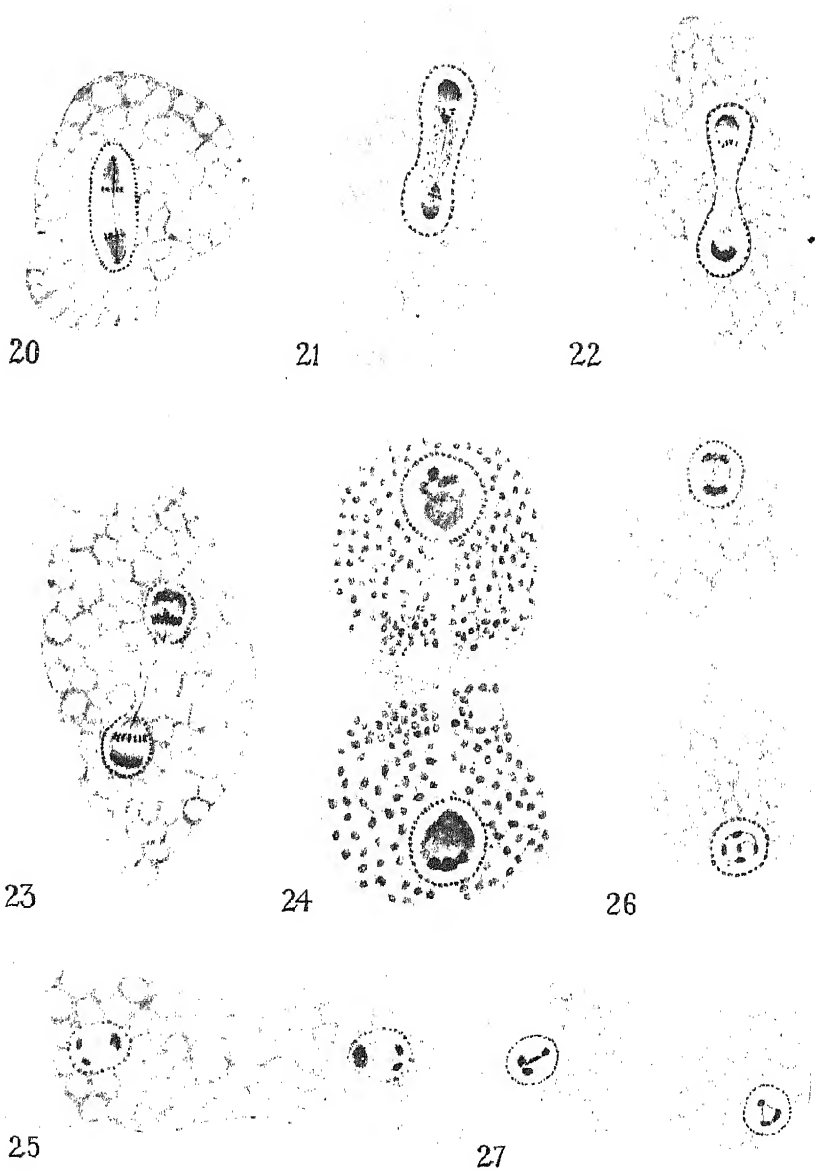


PLATE 3

EXPLANATION OF FIGURES

- 20 Typical anaphase. Polar granules and centrodesmose well differentiated.
Figures 21 and 22 Late anaphase or early telophase.
- 21 Constriction has begun; central part of spindle somewhat ehromatic; daughter plates of chromosomes clumped; centrodesmose visible.
- 22 Constriction more pronounced; daughter groups of chromosomes less clumped; centrodesmose showing.
- Figures 23 and 24 Telophases with constriction completed and a portion of spindle remaining outside daughter nuclei.
- 23 Daughter chromosomes still distinct.
- 24 Nuclear division completed; plasmotomy has started.
- Figures 25 to 27 Plasmotomy completed; partial reorganization of daughter nuclei.
- 25 Daughter nuclei completely rounded up; no spindle remnants; constriction of cytosome has begun.
- 26 Constriction of cytosome almost completed.
- 27 Two daughter amoebae which have just separated.

PLATE 4

EXPLANATION OF FIGURES

28 Early prophase (chromacetic); caryosome resolved into many chromophilic granules.

Figures 29 to 32 Unusual granular spindles.

29 Elongated nucleus beginning to constrict; all spindle elements masked by granules.

30 Stage similar to figure 29; early anaphase plates of chromosomes visible.

31 Granular spindle with large polar masses; other spindle elements masked.

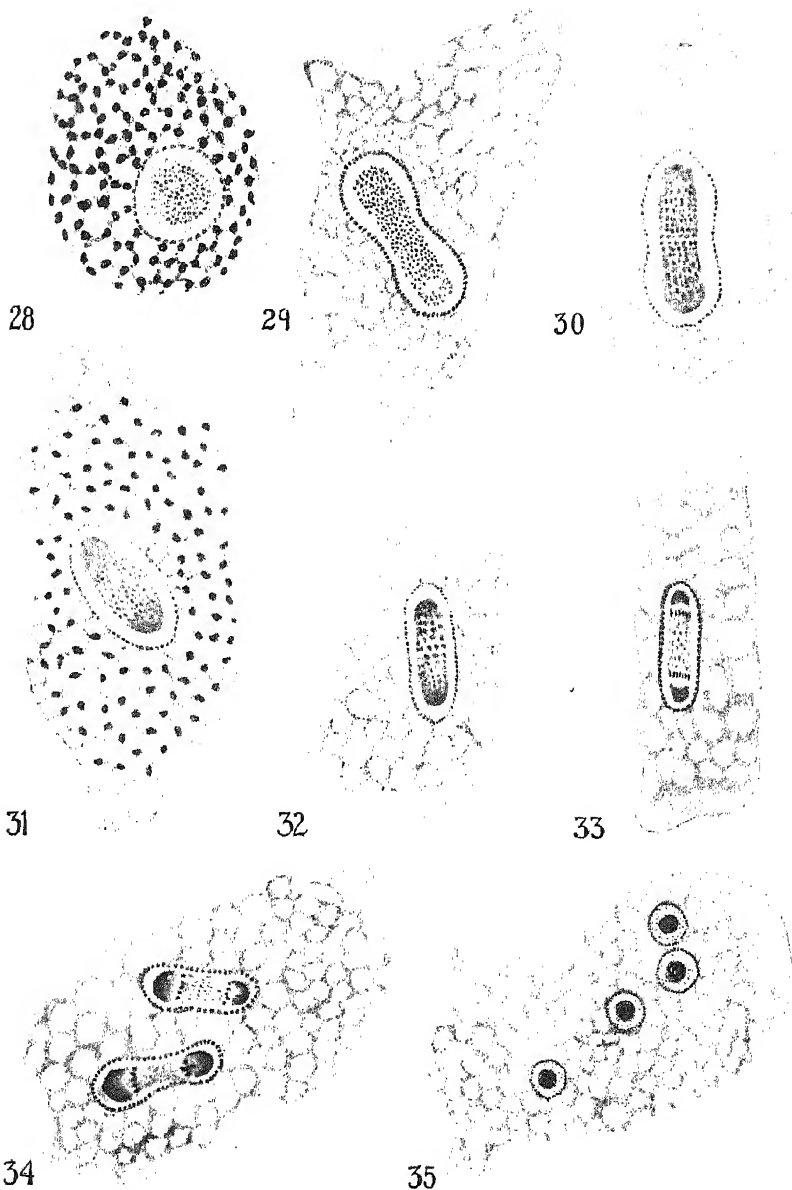
32 Smaller spindle similar to figure 30.

33 Anaphase with many granules in middle part of spindle.

Figures 34 and 35 Multinucleate amoebae.

34 Two nuclei; anaphase of division.

35 Four nuclei in 'resting' state.



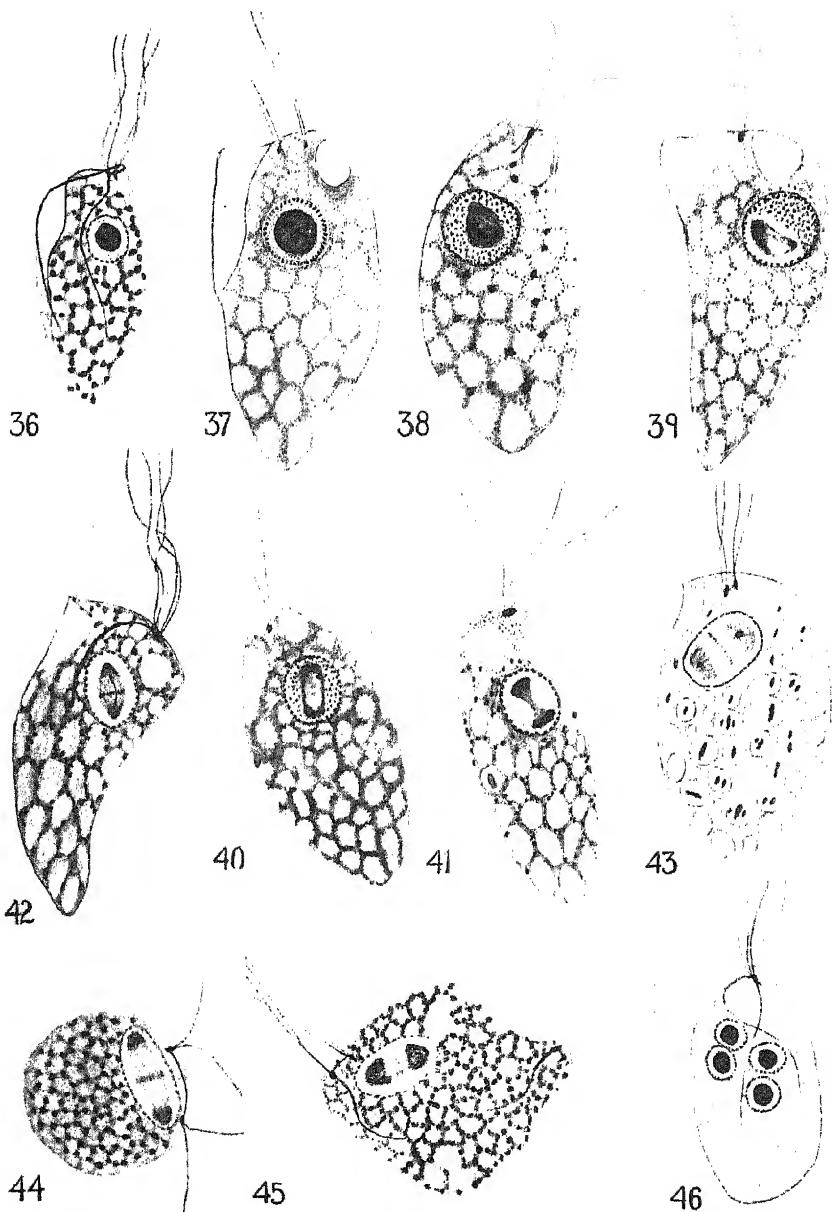


PLATE 5

EXPLANATION OF FIGURES

36 Flagellate phase with nucleus in 'resting' condition; blepharoplast paired; rhizostyle and cytostomal fibrils shown.

Figures 37 to 42 Flagellates with nuclei in prophases.

37 Early prophase; nucleus enlarged; caryosome reticulate.

38 Early prophase; pericaryosomal granules more evident.

39 Early prophase; pericaryosomal granules collected at one side.

40 Oblong caryosome with vacuole-like space in center.

41 Caryosome dumb-bell-shaped; pericaryosomal granules collecting in equatorial region.

Figures 42 to 45 Metaphases.

42 Small spindle formed; polar masses and centrodesmose differentiated.

43 Polar masses large; polar granules and centrodesmose well shown.

44 Nucleus and spindle much elongated; polar masses definite.

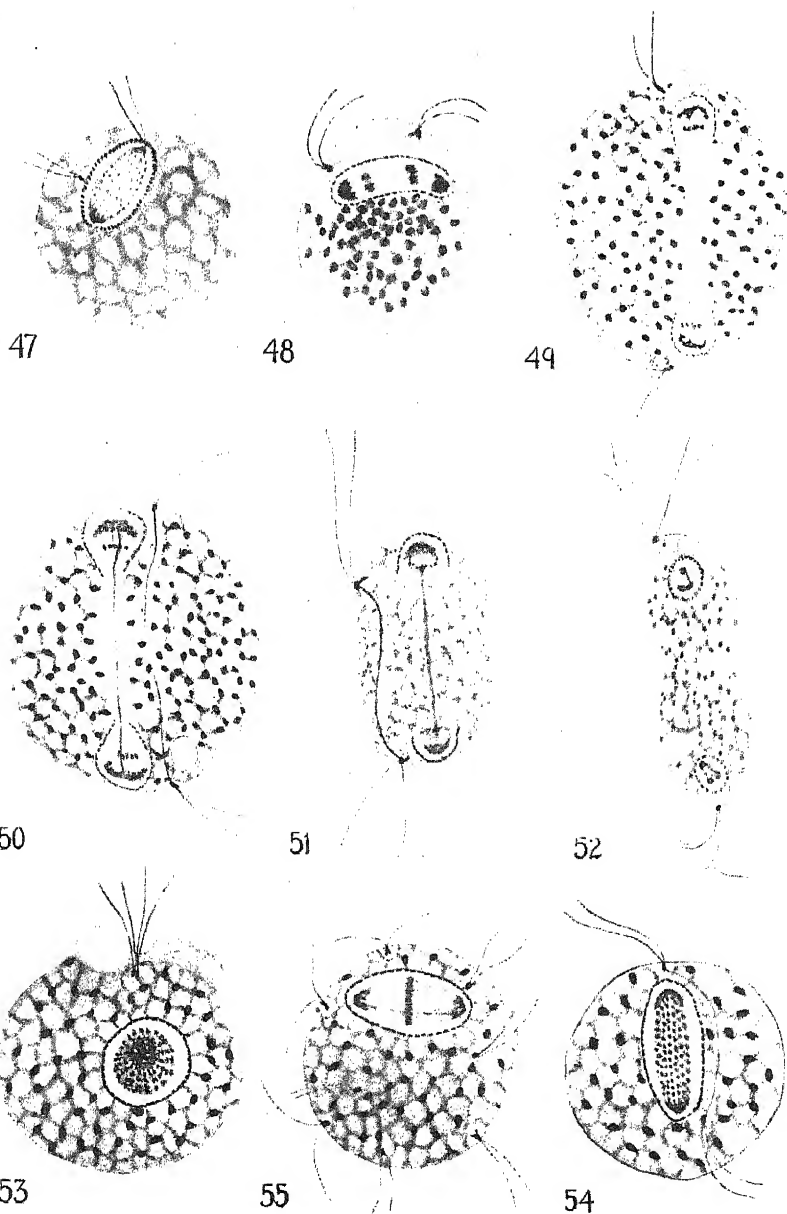
45 Animal with four blepharoplasts; dividing nucleus shows large polar masses and centrodesmose.

46 Flagellate with one blepharoplast and four nuclei.

PLATE 6

EXPLANATION OF FIGURES

- 47 Flagellate with granular spindle like that in figure 11, probably metaphase.
48 Anaphase. Chromosomes clumped; daughter blepharoplasts well separated.
Figures 49 to 52 Telophases.
49 Nuclear constriction well advanced; daughter plates of chromosomes fairly distinct.
50 Longer spindle; constriction well advanced; daughter chromosomes distinct; paradesmose parted.
51 Daughter nuclei rounding up, leaving portion of spindle outside.
52 Daughter nuclei completely rounded up; four flagella on each blepharoplast; cytosome beginning to constrict.
Figures 53 and 54 Flagellates with granular spindles comparable to those in figures 29 and 30.
53 Polar view of spindle. Pseudopodium formed, indicating impending transformation into an amoeba.
54 Side view. Spindle elements masked by granules.
55 Flagellate with eight daughter blepharoplasts and one dividing nucleus.



THE HISTORY OF THE CHROMOSOMAL VESICLES IN THE SEGMENTING EGG OF CRYPTOBRANCHUS ALLEGHENIENSIS

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SIX PLATES (FIFTY-EIGHT FIGURES)

AUTHOR'S ABSTRACT

During the telophases each chromosome becomes inclosed in an individual sac or vesicle which, together with its contents, is called a chromosomal vesicle. The vesicular membrane is of cytoplasmic origin, but is formed under the influence of the chromosome and a droplet of karyolymph. A precise numerical correspondence between chromosomes and chromosomal vesicles has not been established, but it is evident that most, if not all, of the chromosomal vesicles retain their individuality during the resting stage and until after the new chromosomes have been fully formed.

The transformation of the telophase chromosome into the reticulum of the resting stage and the manner in which a new chromosome is formed from a portion of this reticulum are described in detail. In the early prophases each developing chromosome is embedded in a sheath or matrix of less deeply basophilic material, which disappears before the middle prophase is reached.

The formation of chromosomal vesicles is interpreted as a device for doing more rapidly and effectively, under stress of special circumstances, the work that the nucleus must accomplish during the so-called resting stage.

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INTRODUCTION

The idea of the genetic continuity of individual chromosomes from generation to generation of cells derived by mitosis forms an indispensable part of the chromosome theory of heredity. In view of the importance of this conception, the direct evidence in favor of chromosomal continuity is not so abundant as could be desired. The great obstacle in the way of a complete demonstration of the essential continuity of individual chromosomes throughout the nuclear cycle lies in the fact that the chromosomes, as such, are lost to view during the so-called resting stage of the nucleus. The most promising material for the study of this obscure period of the nuclear history appears to be that in which each chromosome, during the telophases, becomes inclosed in a single sac or vesicle which, together with its contents, is called a chromosomal vesicle. Although the occurrence of chromosomal vesicles in segmenting eggs has been noted by a large number of authors working with many different forms, in most cases these vesicles have received merely incidental mention accompanied by inadequate illustrations. Only a few workers, notably Conklin ('01 and '02), Richards ('17), Kater ('26), and McNabb ('28) have made a special study of the history and the significance of chromosomal vesicles.

Mention has already been made (Smith, '19) of the occurrence of chromosomal vesicles in the cleavage stages of the egg of *Cryptobranchus allegheniensis*. Here the chromosomal vesicles of a single nucleus are compactly arranged to form two closely apposed groups of maternal and paternal origin, respectively. For the study of chromosomal vesicles, the *Cryptobranchus* material possesses some marked advantages, as well as some obvious disadvantages. For a demonstration of constancy in the number of chromosomes and chromosomal vesicles, one must seek material in which the vesicles are more loosely arranged or in which the number of chromosomes is much smaller; but in the blastomeres of *Cryptobranchus* the large size of the nucleus, the absence of yolk in its immediate vicinity, and the entire absence of pigment are circumstances

favoring the study of the intimate structure of the chromosomal vesicles.

MATERIAL AND METHODS

The division of the cleavage period of *Cryptobranchus* into stages has been described and illustrated in earlier papers (Smith, '12 b and '26). I have divided the entire period of cleavage into ten stages, of which stages 1 to 6, inclusive, represent each a single cell generation, while each of the later cleavage stages comprises several cell generations. Chromosomes, chromosomal vesicles, and entire nuclei are largest in the early stages of cleavage, becoming progressively smaller as the blastomeres become more numerous. For the study of nuclear structure, stages 3 to 8, inclusive, are the most favorable, since in these stages each nucleus is surrounded by a large mass of yolk-free cytoplasm. In stages 1 and 2 the nuclei, though very large, are few in number and sometimes partially obscured by yolk granules; in stages 9 and 10 the nuclei are comparatively small and are usually more or less obscured by yolk.

Observations were chiefly confined to the nuclei of micromeres, since these nuclei are more numerous and generally better preserved than the nuclei of macromeres. Horizontal sections are preferable to vertical sections, since horizontal sections more often furnish nuclei favorably oriented. All the drawings used to illustrate this paper were made from horizontal sections through the region of micromeres. About 200 eggs, representing all stages of cleavage, were studied in serial sections; nearly all the nuclei of micromeres in these eggs were examined under high powers of the microscope and a large number of them were made the objects of intensive study.

Most of the material used was fixed in a bichromate-acetic-formalin mixture, the formula for which has already been published (Smith, '12 a and '26). Further experience with this fixing fluid has shown that the best results are obtained when the eggs are immersed in the fixing fluid for a period of

thirty-six to forty-eight hours, provided the stock solution of bichromate-acetic (to which formalin is added at the time of using) is reasonably fresh. The only other fixing solution that has been found to preserve the form, as well as the finer structure, of the blastula is a sublimate-acetic-formalin mixture which gives inferior results. All the drawings were made from eggs fixed in the bichromate-acetic-formalin mixture.

An important consideration affecting cytological work is that the material should not be stored too long in formalin or in alcohol before it is embedded in paraffin preparatory to sectioning. Most of the material used was embedded in paraffin about six weeks after preservation; it was sectioned, stained, and mounted in balsam about six months later. Fair results were obtained with *Cryptobranchus* material that had been stored in alcohol for periods of time varying from one to four years; material stored in alcohol longer than this was usually worthless for the study of the finer details of structure.

For a study of the various structural features of the large nuclei of the segmenting egg of *Cryptobranchus*, it is essential that an adequate number of eggs shall be cut in series of various thicknesses, ranging from 5μ to 25μ . For a study of the general form and topography of the nucleus, thicknesses of 10μ to 12μ are best, while for chromosome counts thicknesses of 15μ to 25μ are required. For a study of some of the intimate details of structure in the late telophases, resting stage, and very early prophase, sections 5μ to 8μ thick are satisfactory.

All the sections were stained with Heidenhain's iron haematoxylin, usually without a counterstain. Unless a counterstain is specifically mentioned, the descriptions are based on sections stained with iron haematoxylin alone. A considerable number of sections were counterstained with Bismarck brown, but very little additional information was gained by its use. This accords with the writer's previous experience with the use of counterstains after iron haematoxylin: in general, they tend to obscure the finer features of nuclear structure and

are objectionable unless used sparingly and with very thin sections. Bismarck brown stains beautifully the asters and spindle fibers, but these are readily visible without a counter-stain. In the resting stage and early prophases Bismarck brown stains the nuclear sap and thus tends to obscure the delicate strands of the reticulum. All the drawings were made from sections stained with iron haematoxylin alone.

For the routine study of these preparations, the writer has used chiefly a Zeiss apochromatic objective 60 (3 mm.) with a Zeiss compensating ocular $10\times$ (no. 8). With these lenses, even such delicate structures as the reticulum of the resting stage and early prophases are brought out with a clearness quite comparable to that obtained with any of the considerable assortment of higher-power lenses that were available.

In making the outlines for drawings, a Zeiss achromatic objective 100 (1/12 Fl., semi-apochromatic) was used with a Zeiss Huyghens eyepiece $7\times$ (no. 3); this combination gives a magnification of 700 diameters. All the figures were drawn with the aid of a camera lucida, which increased the magnification to 1500 diameters. Figures 41 and 47 were afterward enlarged three diameters, and figure 48, A to E, was enlarged five diameters. In reproduction all the figures were reduced one-half and thus appear with magnifications ranging from 750 to 3750 diameters. In comparing the figures with respect to size, it should be noted that they are grouped in sequence according to the phases of mitosis, regardless of the stage of cleavage. In late cleavage stages the nuclei are considerably smaller than in early cleavage stages; other differences in the size of the figures depend on the magnification, the phase of the nuclear cycle, the plane of the section, or the depth of the cut through the nucleus. In the few instances where a nucleus is complete in a single section, the fact is mentioned in the explanation of the figures.

In a previous paper (Smith, '19) attention was directed to the duplex character of the nuclei, and illustrations were based on sections of nuclei cut in a plane favorable for revealing this double structure. In the present paper attention is

concentrated on the chromosomal vesicles, and their segregation into two groups is a matter of secondary importance. Incidentally, some of the figures do show the segregation of maternal and paternal chromosomes or chromosomal vesicles, but the selection of material for the illustrations has been guided by other considerations.

I am indebted to Dr. H. D. Senior for a very helpful criticism of the manuscript.

NOMENCLATURE

In the present paper the term chromosomal vesicle is used to designate not only the sac or vesicle that forms around each individual chromosome, but also the contents of the vesicle with special emphasis on the metamorphosed chromosome. The chromosomal vesicle is a unit of nuclear structure. The term karyomere has often been used as a synonym, but there are conditions of the nucleus in which it is divided into parts that might appropriately be called karyomeres, yet these parts do not represent single chromosomal vesicles. Thus, in the cleavage stages of *Cryptobranchus*, each resting nucleus ordinarily consists of two compact groups of chromosomal vesicles, each group representing a complete assortment of chromosomes derived from one of the parents. In the late cleavage stages irregularities occur, in that the entire nucleus may consist of three or more groups of chromosomal vesicles, each group so compact that in a cursory observation it appears to be a single vesicle.

In describing the late telophases, resting stage, and early prophase difficulty was experienced with the usual nomenclature, owing to the fact that the differentiations which occur during the metamorphosis and reconstruction of a chromosome appear to involve only slight modifications of the same fundamental substance, and some of these changes are reversible. Identical forms may appear with different staining reactions, and vice versa. In particular, there is no clear-cut and enduring distinction between the substances to which the

terms chromatin and linin are applied, so long as these terms are used in a mutually exclusive sense. This difficulty is partially overcome by using the term chromatin to designate the entire substance of the chromosome and the structures derived from it through a process of metamorphosis. In this sense, both the reticular framework and the more compact masses, nodules, or spherules associated with it are composed of chromatin, regardless of whether these substances are basophilic or oxyphilic. It is convenient to retain the term linin to designate the strands of the reticulum, so long as these are oxyphilic. The chromatin nodules or spherules exist in three phases, as explained in the account of the late telophases, hence the mere designation of these nodules as basophilic or oxyphilic is not entirely sufficient for purposes of description.

OBSERVATIONS

During early cleavage there is a close synchronism in the occurrence of mitosis in the blastomeres of a single egg; all the nuclei are in pretty nearly the same phase. In middle cleavage the macromeres lag behind the micromeres and there is a fine gradation between the phases represented by the smaller and the larger micromeres, respectively. In late cleavage practically all phases of mitosis may be found in the micromeres of a single egg, but for reasons already stated the late cleavage stages are not so satisfactory for the study of nuclear structures.

The observations here recorded are based on the study of eggs in all stages of cleavage, although stage 10 was not studied so extensively as the earlier stages. Aside from a gradual decrease in the size of the nucleus, there appears to be no important difference in the nuclear changes correlated with the stage of cleavage, save an apparent tendency for the individual chromosomal vesicles to fuse with each other to some extent during the very late cleavage stages.

Though this account is primarily concerned with the chromosomal vesicles, it seems advisable to begin with a phase during which the individuality of the chromosomes is best

expressed. One must learn something of the number, form, size, and arrangement of the chromosomes before proceeding to trace their transformation into chromosomal vesicles. The chromosomes are fully formed in a middle prophase, but at this time the situation is complicated by the presence of a linin network and the disintegrating walls of the chromosomal vesicles. The splitting of the chromosomes usually begins during a very late prophase, but the actual separation of the halves is accomplished during the metaphase and the anaphases. A late prophase would seem to be the logical place to begin the study of the nuclear cycle, were it not for the fact that in this phase the arrangement of the chromosomes is still somewhat diffuse, so that it is difficult to obtain a comprehensive view in a single section. It therefore seems advisable to begin with nuclei just entering the metaphase.

1. The metaphase (figs. 1 to 7)

The term metaphase is here used to designate the phase in which the chromosomes are arranged approximately in the equatorial plane of the spindle, midway between the poles. In this phase it is possible, in sections of the proper thickness, to obtain polar views of nuclei in which all the chromosomes are either represented or entirely contained in a single section.

In a polar view of an early metaphase the chromosomes are seen to be of various sizes. They are usually arranged in the general form of a rosette (fig. 2). At the periphery of the group, long irregularly V-shaped chromosomes extend in a somewhat radial direction, with the apex of the V pointing toward the axis of the spindle. In the center of the group very short rod-shaped chromosomes usually predominate, while chromosomes of intermediate sizes, some rod-shaped, some hook-shaped, are scattered throughout the entire nucleus. The number of chromosomes will be considered in a later section, but it is evident from a casual inspection of the figures that the number is much larger than is typical for urodeles.

In the stage of transition from prophase to metaphase (figs. 1 and 2) it may be seen by careful inspection that all, or nearly all, the chromosomes are constricted lengthwise, but the halves are closely adherent throughout and the double nature of the chromosomes is not apparent in the figures. In the longer chromosomes it often happens that the halves wind spirally about one another. During the metaphase the halves of the longer chromosomes begin to separate, first at the ends, then by a gradual loosening of the attachment throughout their length, and finally by pulling apart at the middle where a spindle fiber is attached to each segment. The short rod-shaped chromosomes split apart in simpler fashion and are completely separated during the metaphase, but the longer chromosomes remain hooked together at their ends until their complete separation marks the beginning of the late anaphases.

During the metaphase a few pale and swollen fragments of the degenerating walls of chromosomal vesicles of the parent nucleus persist among the chromosomes, but these fragments are more readily recognizable after one has studied the complete structures in the late telophase, resting stage, and early prophase. The fragments are not shown in the drawings of the metaphase.

During the metaphase there appears to be little, if any, undifferentiated cytoplasm in the intervals between chromosomes. These intervals are traversed by very numerous spindle fibers which branch and anastomose; the branches bear a considerable resemblance to the strands of cytoplasm that form a reticulum in regions remote from the nucleus, but differ in that the spindle fibers are straighter and more regularly arranged. Most of the structures that look like granules in both the spindle fibers and the undifferentiated cytoplasm are optical sections of strands extending in the line of vision.

2. The anaphases (figs. 8 to 16)

In these phases the separation of the chromosomes into two daughter groups becomes complete and the groups move

apart toward opposite poles of the spindle. In the movement toward the poles the smallest chromosomes precede, perhaps because they are more readily separated from their sister chromosomes. The longer rod-shaped chromosomes assume a position approximately parallel to the long axis of the spindle. The hook-shaped chromosomes of each group lie with their hooked ends directed away from their sister chromosomes toward opposite poles of the spindle. The long V-shaped chromosomes are the last to become completely separated from their sister chromosomes; in this separation the middle portion or apex precedes, while the ends of each chromosome remain for a long time hooked or coiled about the ends of its sister chromosome (figs. 8 to 11). The limbs of these long V-shaped chromosomes, which were previously often coiled about their sister chromosomes, become straight, as if drawn taut, save at their ends, which remain coiled where still in apposition with their sister chromosomes. The ends straighten as they release their hold, and the other portions of the chromosome then sometimes become slightly kinked, as if released from tension (figs. 11 to 13). After the V-shaped chromosomes are completely separated from their sister chromosomes, their limbs usually become approximately parallel, so that the general form is more nearly like that of an elongated U. This change of form of the V-shaped chromosomes is part of a general concentration of the chromosomes of each daughter nucleus into a more compact group, which takes place during the late anaphase (figs. 13 to 16). All the chromosomes come to lie parallel to one another and to a line connecting the two centrospheres; the smaller chromosomes of each daughter nucleus are nearer the pole of the spindle.

During the early anaphases each chromosome, if carefully stained and examined under high magnification, shows a finely zigzag or spiral contour; its appearance often suggests the presence of a zigzag or spiral filament (chromonema of Vejdovský) consisting of material more deeply basophilic than the remainder of the chromosome. The existence of such a

structure was not clearly recognized during the present study. In the late anaphase, when a chromosome has been heavily stained with iron haematoxylin and then strongly destained in the iron alum, the chromosome may be seen to consist of masses of deeply stained material alternating with regions that are decidedly pale. The deeply stained masses usually occur at regular intervals, either as nodules extending the entire width of the chromosome and giving it a beaded appearance, or as oblique bands giving the impression of a spiral filament; but some of the dark masses are crescentic in outline and a few are irregular. Sometimes, two exceptionally small dark masses occur on opposite sides of a chromosome. Some of these dark masses are connected in series by slender basophilic bridges, but most of them are discontinuous. It is possible that these dark masses are formed by the fragmentation of an originally continuous zigzag or spiral filament, but in any case the condition observed in the late anaphases represents a preparation for the changes that occur during the metamorphosis of the chromosomes after the vesicles have been formed.

In the early anaphases there is a considerable amount of undifferentiated cytoplasm mingled with the spindle fibers between and around the individual chromosomes. This undifferentiated cytoplasm is distinguishable from the spindle fibers, which are more regular in their distribution. In the later anaphases, owing to concentration of the chromosome groups, the spindle fibers become more crowded, so that together with the undifferentiated cytoplasm they form a compact feltwork between and around the individual chromosomes. As will presently appear, this is a condition favoring the formation of the chromosomal vesicles.

During the anaphases a few swollen and transparent fragments of the walls of the chromosomal vesicles of the parent nucleus are sometimes left in the equatorial region. These fragments are not represented in the figures.

3. *The telophases* (figs. 17 to 34)

The telophases comprise a series of events involving profound changes in the chromosomes. The first of these events is the formation of an extremely thin vesicular wall inclosing each individual chromosome, which meanwhile becomes spirally coiled within the vesicle. Each vesicle then increases in size, as if distended by osmotic pressure, and the chromosome becomes metamorphosed into a reticulum in which the coarser strands and nodules indicate the site of the original chromosome. Each vesicle together with its contents is a chromosomal vesicle. It is evident that the vesicular walls, if maintained during the resting stage, prevent the mingling of chromatin belonging to different chromosomes.

The smallest chromosomes are the first to become inclosed within vesicles. In the case of the long U-shaped chromosomes, the vesicular wall forms rapidly about the middle or looped portion of each and the adjacent portions of its limbs, then proceeds more gradually to inclose the free ends which are directed toward the equator of the spindle.

The manner in which the vesicular wall is formed is more readily perceived in relation to the smallest chromosomes. Attention is first attracted to a clear rounded space on one side of a chromosome, between the chromosome and the meshwork of cytoplasmic strands. At first, this clear space is bounded only by the chromosome and by strands of cytoplasm, as if the cytoplasmic meshwork had been pushed away from the chromosome by the accumulation of a viscous fluid; but wherever the cytoplasmic strands at the margin of the clear space come into contact or close proximity with the chromosome they become thicker and more opaque. As soon as the clear space becomes large enough to surround the chromosome, the latter becomes curved or slightly coiled within the clear space, and a continuous membrane forms at the boundary of the clear space, except on the side opposite the concavity of the chromosome. The vesicular wall or membrane is formed by the coalescence of rather deeply staining

plates originating as a modification of the thickened strands of cytoplasm bounding the clear space and in close proximity to the chromosome. The outer curvature or convex side of the chromosome becomes closely adherent to the inside of the vesicular membrane, which is best developed in the vicinity of the chromosome and frays out to become continuous with ordinary strands of cytoplasm on the side farthest from the chromosome. In sections taken in a favorable plane, the vesicular membrane in this stage appears U-shaped or C-shaped, with the chromosome lying on the side farthest from the gap in the vesicle (figs. 17 to 20). In the light of later developments, it is evident that the clear space represents a droplet of karyolymph or nuclear sap which has formed at the surface of the chromosome and, coming in contact with the cytoplasm, has influenced the formation of a barrier to the further diffusion of the karyolymph. That the chromosome is directly or indirectly a factor in supplying the stimulus for the formation of the vesicular wall is evident from the fact that the part of the wall nearest the chromosome is the first to form. The gap in the vesicular membrane is later closed (fig. 21) by an extension of this membrane, apparently at the expense of the cytoplasm.

Excepting at their free ends, the long U-shaped chromosomes become undulating before vesiculation commences. One or more small clear spaces appear alongside each chromosome, each space lying against a concavity formed by the chromosome. These clear spaces enlarge to include a portion of the chromosome, coalesce where they come in contact with one another, and become bounded by a vesicular wall in the manner described for the very small chromosomes. Early stages in the formation of the vesicular wall, when the wall is incomplete on the side farthest from the chromosome, have been observed in transverse sections of the long chromosomes, as well as in entire long chromosomes (figs. 18 and 19). In later stages the vesicle formed about a portion of a long chromosome enlarges until it surrounds the entire chromosome (figs. 20 to 27).

Since the telophase chromosomes occur in a region of abundant spindle fibers whose anastomosing branches are now almost indistinguishable from strands of undifferentiated cytoplasm, it is not easy to determine whether the vesicular membrane forms entirely from ordinary cytoplasm or partly from spindle fibers; but, if the membrane is examined in sufficiently early stages, it becomes obvious that it is not derived from the chromosome.

As shown by the figures, the area finally inclosed by the vesicular membrane is considerably larger than the chromosome, hence attention was directed to the possibility that some cytoplasm might be inclosed within the vesicle. But, in the stage just before the vesicular wall becomes complete, the area that seems destined to be inclosed is free from cytoplasm. Direct inspection of the newly completed vesicle shows that it contains only the chromosome and a clear fluid.

In the early stages of its formation the vesicular membrane stains at first faintly, then distinctly, with iron haematoxylin, and is never highly refractive. When complete, the vesicular wall seems more resistant to this stain and often remains unstained; it is then highly refractive. The vesicular membrane does not stain readily with Bismarck brown except during the early and middle prophases.

In all cases the chromosome becomes more or less coiled or convoluted before the vesicular wall is complete; each of the longer chromosomes eventually assumes the form of a loose spiral. This coiling adapts the chromosome to the form of the vesicle, which tends to be spherical. The vesicles formed around the shortest chromosomes become at once spherical, but those formed around the longest chromosomes attain this condition only gradually and with some difficulty, owing to their mutual relations. The vesicles formed about the long U-shaped chromosomes are at first somewhat U-shaped, then club-shaped, flask-shaped, or retort-shaped (figs. 23 to 27); their forms and relations are best observed in thick sections.

So long as the chromosomes remain intact, the vesicles ordinarily contain no other structures; the clear space within

the vesicle affords a marked contrast to the cytoplasm without. During the early telophases the karyolymph remains unstained by Bismarck brown. Iron haematoxylin does not stain the karyolymph at any stage. The chromosomes or portions of chromosomes first included within vesicular walls are the first to undergo further changes.

Owing to the loose manner in which the chromosome is coiled, the chromosomal vesicle enters the middle telophases (figs. 24 to 33) with most of the chromosome occupying a peripheral position within the vesicle. At the beginning of the middle telophases, the chromosome is broken up into a series of rod-shaped segments of variable length, connected end to end by a delicate strand of linin. Occasional cross branches of linin connect chromosome segments belonging to adjacent limbs of a coil, and a few long slender strands of linin may extend completely across the vesicle, connecting chromosome segments lying on opposite sides of the vesicle. A little later, each chromosome segment assumes a tattered, somewhat vacuolated appearance and stains less deeply with iron haematoxylin; it becomes broken up transversely into irregular fragments connected by short stout strands, and may send out short side branches. During the late telophases each modified chromosome segment becomes resolved into a sharply defined zigzag strand, slightly stouter than the linin strands and slightly basophilic; at its angles occur small rounded nodules of basichromatin (figs. 34 to 41). Apparently not all the chromatin of the chromosome segments is used up in the formation of the zigzag strand and its nodules, for, after these structures are fully formed, irregular fragments of disintegrating chromatin may be found attached to them. Meanwhile, there is being built up a reticulum of linin strands (fig. 41) branching out from the coarser zigzag strands and likewise occupying a peripheral position within the vesicle. The linin strands are at first exceedingly delicate, but they become slightly stouter and bear minute oxyphilic spherules. Some of the coarse zigzag strands branch and anastomose to form a small reticulum readily distinguishable

from the paler and more delicate linin reticulum with which it is continuous. As shown by a comparison with the early resting stage, the coarser portion of the reticulum increases by the transformation of linin strands and oxyphilic nodules into basophilic strands and nodules.

During the middle and late telophases a few rather large pale rounded granular masses may be found variously distributed within the vesicle. Some are loosely attached to the coarser strands of the reticular framework; some are attached at fairly regular intervals along rather attenuated strands of linin, and some are free within the vesicle. In the drawings these granular masses, which are more numerous during the resting stage and very abundant in the early pro-phases, are represented by circular outlines that fail to portray their granular appearance. When stained with iron haematoxylin alone, the granular masses vary in staining capacity from dark to very light, but they are usually pale. The granular appearance is more marked in the masses which are free than in those attached to the reticulum; the latter bear a closer resemblance to ordinary chromatin nodules. There seems no reason to doubt that these masses represent degenerating chromatin derived either from basichromatin nodules or from particles of chromatin detached when the chromosome segments are resolved into zigzag strands. During the late telophase the karyolymph stains slightly with Bismarck brown.

As previously stated (Smith, '26), the centrosomes are rarely distinguishable in sections stained with iron haematoxylin, but the centrospheres are large and conspicuous. At the beginning of the late telophases, when the vesicles are fully formed and the chromosomes are beginning to segment, the centrospheres divide. In the stage of transition from telophase to resting stage, each daughter nucleus is accompanied by two centrospheres located on nearly opposite sides of the nucleus.

4. *Interkinesis, or the resting stage* (figs. 42 to 44)

Here the chief point of interest lies in the fact that most, if not all, the walls of the chromosomal vesicles persist during the entire resting stage and into the early and middle pro-phases. Until the chromosomal vesicles are counted and compared with the number of chromosomes in the division phases, one cannot be sure that every chromosomal vesicle maintains itself as a separate entity until the new chromosome is fully formed within it, but various considerations make this probable. There is no nuclear membrane aside from the walls of the chromosomal vesicles. The topographical arrangement of vesicles of various sizes, corresponding to the arrangement of chromosomes in the late anaphases, is not much changed during the resting stage. While the number of vesicles recognizable in serial sections seems somewhat smaller than the number of chromosomes in the division phases, this is readily accounted for by the fact that, in places where vesicles overlap, the density of the chromatin content makes dividing walls difficult to recognize. Complete reconstructions of the chromosomal vesicles of entire nuclei are impracticable.

In the resting stage the reticular framework is best developed near the periphery of the vesicle, where it is distributed rather uniformly. During the early resting stage (figs. 42 and 43) this reticular framework is not of the same character throughout. In some regions the reticulum is comparatively coarse; its slightly basophilic strands bear numerous deeply basophilic nodules not only at intersections, but often at intervals between intersections. Alternating with these regions are others in which the reticulum is more delicate, consisting of thin strands with occasional small oxyphilic spherules. The coarser portions of the reticulum occur in elongated, branching, and spirally curving tracts whose distribution often makes it evident that they represent the main body of the metamorphosed chromosome; this condition is not well shown in figures 42 and 43.

The framework of linin strands is of such delicacy that one despairs of representing its true appearance in pen-and-ink drawings at the magnifications employed. In the figures the linin strands are represented by dotted lines or cords, but their real appearance is that of very slender pale homogeneous filaments bearing occasional small pale swellings or spherules. At the borders of the coarser portion of the reticulum there is every gradation in size and staining reaction between the smallest and palest spherules and the smaller basichromatin nodules. The basichromatin nodules are of various forms: the smaller ones are usually spherical and the larger ones are elongated in the direction of the axis of a linin strand to which they are attached. It is evident that some, at least, of the basichromatin nodules are developed from oxyphilic spherules.

During the late resting stage (fig. 44), along with an increase in the size of the entire vesicle, there is an increase, relative as well as absolute, in the extent of the portion of the reticulum bearing basichromatin nodules and a corresponding decrease in the extent of the more delicate portion of the reticulum. This change comes about through the transformation of oxychromatin spherules into basichromatin nodules, and is accompanied by a slight thickening of the linin strands. On the other hand, the slightly basophilic strands which connected the basichromatin nodules of the early resting stage have become oxyphilic; therefore, the entire framework now consists of linin. Thus, in the late resting stage, there is a closer approach to uniformity in the reticular framework, as if a partial compromise had been reached between the characteristics of the two regions distinguished in the early resting stage.

During the resting stage there is a perceptible increase in the number of the large pale granular masses that have already been described in the late telophase and interpreted as degenerate chromatin nodules. In the resting stage these masses occur, in about equal numbers, either free within the vesicle or attached to strands of the reticulum. During the

resting stage the karyolymph stains moderately with Bismarck brown, tending to obscure the linin strands.

All the chromosomal vesicles of a single nucleus do not enter or leave the resting stage quite synchronously. When most of them are in the resting stage, some may be in a very late telophase, or some may be in a beginning prophase. Different parts of a large chromosomal vesicle may exhibit a brief sequence of phases. The lagging transformation of the large chromosomes, particularly at their free ends, has been noted in the telophases, and this may serve to explain the differences noted during the resting stage.

The chromosomal vesicles increase in size during the resting stage and the maximum size of the nucleus is not attained until an early prophase. During the resting stage the centrospheres arrange themselves on opposite sides of the vesicular nucleus, which undergoes rotation in the manner described in a previous contribution (Smith, '19).

5. *The prophases* (figs. 45 to 58)

The early prophases (figs. 45 to 50) comprise the period of the formation of new chromosomes. In general terms, the formation of a prophase chromosome consists in the thickening of a single strand of linin comprising a superficial portion of the reticulum carried over from the resting stage. The entire strand winds about in large loose folds or convolutions, often in spiral fashion, in such a manner that all parts of the strand remain at or near the periphery of the vesicle, just within the vesicular wall. In the earliest stages of its differentiation from the reticulum, this thickened strand retains a fine zigzag structure imposed upon it by its origin from the reticulum; its basichromatin nodules are situated at the angles of the strand and many of them retain their connections with adjacent portions of the reticulum (figs. 45 to 48, A). In these early stages of its development, the thickened strand that is to form the new chromosome is already conspicuous by reason of the presence of a sheath or matrix best developed around the nodules, but gradually extending

over the internodes; this matrix is composed of material only slightly less basophilic than the nodules from which it seems to be derived. As the thickened strand develops (fig. 48, B to C), its angles are somewhat smoothed out, so that it becomes finely undulating, often in the form of an elongate spiral; its internodes gradually become more basophilic, while its nodules become reduced in size until they disappear as such. Thus the thickened strand becomes a filament of nearly uniform diameter and staining capacity, finely coiled like a corkscrew, though not always so regular in form; this filament becomes deeply basophilic. Meanwhile, the sheath or matrix becomes distributed more uniformly along the surface of the filament and in the spaces between its coils, so that the entire structure becomes approximately rod-shaped (fig. 48, C). In slightly overstained preparations the sheath or matrix stains with iron haematoxylin so deeply as to obscure the spiral filament, and the entire structure looks very much like a completed chromosome of the middle and late pro-phases. Near the close of the early pro-phases, the sheath or matrix degenerates in situ, its peripheral portion being the last to disappear (fig. 48, D). Before the sheath has entirely disappeared, the deeply basophilic spiral filament has thickened until its spiral windings are no longer so prominent. No part of the sheath or matrix present in the early pro-phases persists to contribute to the formation of the completed chromosome of the middle and late pro-phases; the completed chromosome is developed entirely from the spiral filament. Nevertheless, the completed chromosome (fig. 48, E) bears an imperfect resemblance to an earlier stage in which the spiral filament is embedded in a matrix. In studying a chromosome of the middle pro-phases under high magnification, one often gets the impression that the original slender zigzag or spiral filament is represented by an unusually dense portion (chromonema) embedded in material slightly less basophilic. This appearance may possibly be an optical illusion due to the finely spiral or zigzag undulation of the chromosome as a whole.

It is evident that even the superficial portion of the reticulum is not entirely used up in the formation of a new chromosome. It is significant that in its distribution, general topography, and finer structure a new chromosome just emerging from the reticulum strongly resembles the parent chromosome in a late telophase entering the final stages of its metamorphosis (compare figs. 47 and 48, A, with fig. 41). It seems very probable that the new chromosome is formed out of an axial portion of the reticulum, representing the old chromosome or main stem from which branching and anastomosis occurred. In this way there may be a definite correspondence of part for part, in serial order, between the new chromosome and the old.

The portion of the linin reticulum that does not take part in the formation of the new chromosome gradually loses most of its connections with the new chromosome, recedes more or less from the surface of the vesicle, and is for a time partially obscured by the large pale granular masses that now accumulate in great numbers within the interior of the vesicle (figs. 46 and 49). Many of these granular masses are attached to the reticulum, others are free within the vesicle, and a few may be found attached to the matrix surrounding the new chromosome. The great majority of the masses present in the early prophases are derived from the basichromatin nodules of that portion of the reticulum that does not take part in the formation of the new chromosome; these nodules enlarge and become less deeply basophilic. After the new chromosome is fully formed, the large pale granular masses rapidly become reduced in size and number; at the close of the early prophases, all of them have usually disappeared and the reticulum, which persists, is fully revealed. This reticulum, which represents the portion of the resting-stage reticulum left over after the formation of the new chromosome, still bears numerous small basichromatin nodules (figs. 51 and 52) and a few smaller oxychromatin spherules. During the early prophases the karyolymph stains with Bismarck brown with about the same intensity as the strands of the reticulum.

The middle prophase (figs. 51 to 58) may be defined as the period of partial collapse and almost complete disintegration and absorption of the vesicular walls. The walls separating individual chromosomes are the first to disappear, while the walls separating the two chromosome groups or germ nuclei persist quite as long as the exterior walls. Meanwhile, the linin reticulum bears fewer, smaller, and paler granules until these have all disappeared; but wherever a linin strand extends directly in the line of vision it appears as a sharply defined round dot almost as dark as the basichromatin nodules that it formerly bore. The reticulum then loses its few remaining connections with the chromosome, recedes more uniformly into the interior of the vesicle, and becomes very pale and attenuated before it finally disappears. It is, however, readily distinguishable from spindle fibers even, after the latter have invaded the disintegrating vesicles.

The practical disintegration of the vesicular walls ushers in the late prophases, but the absorption of these walls proceeds slowly, and a few pale swollen fragments are usually recognizable during the late prophases, the metaphase, and sometimes even in anaphases. It is perfectly obvious that the vesicular walls, after a slight collapse, disintegrate in situ; they do not, as might be conjectured, shrink down to form investing membranes or sheaths for the new chromosomes.

During the early and middle prophases the new chromosomes maintain more or less a distribution according to size and shape, similar to that of the telophases. None of the drawings shows an extremely late prophase, but figure 1 represents a nucleus, just entering the metaphase, which retains an arrangement of chromosomes more characteristic of a nucleus in the late prophases. During the late prophases the chromosomes usually undergo a rearrangement leading to their final distribution in the equatorial region in the form of a rosette, as described for the metaphase; each chromosome becomes constricted in a longitudinal plane, preparatory to splitting.

6. *The number of chromosomes*

Definite observations on the number of chromosomes have been confined to the cleavage stages, where of course the diploid number is present. Owing to the large number of chromosomes and to the fact that some of them are very long, the determination of the precise number is a matter of considerable difficulty. Since it is usually impossible to reconstruct the chromosomes from drawings of serial sections, one must use thick sections (15μ to 25μ) and look for those rare cases where all the chromosomes of a nucleus are confined to a single section. In case only a few chromosomes or pieces of chromosomes occur in one of the adjoining sections, their relations may sometimes be determined by careful focusing on the cut surfaces. Yet there is always an element of uncertainty in a chromosome count based on more than one section. It seems possible that, wherever the knife cuts into the nucleus, one or more small chromosomes may be lost in sectioning or during subsequent treatment of the sections; there is also the possibility of fragmentation of a chromosome due to pressure of the knife, leading to counts that are too high. Despite the difficulty of finding complete nuclei even in thick sections, such material has the advantage of giving clearer views than I have been able to obtain in studying a large number of preparations of ectoderm stripped from late embryos and early larvae, and mounted without sectioning.

In polar views of a beginning metaphase the chromosomes may readily be counted, and in some cases it is reasonably certain that none of the chromosomes has divided. The early anaphase, which must be studied in equatorial views, also is favorable for chromosome counts, but in this phase the chromosome groups are diffuse and rarely confined to a single section. It is comparatively easy to find equatorial views of late anaphases in which all the chromosomes are confined to a single section, but in this phase the chromosomes are usually crowded together so closely that it is almost impossible to count them. In all cases the method used was to make a

careful drawing of the chromosomes before attempting to count them.

Owing to the large number of thick sections required, the final determination of the chromosome number is a problem in itself and the meager results recorded here are not conclusive. One nucleus in a beginning metaphase contains 56 chromosomes in one section (fig. 1) and a short rod-shaped piece in an adjoining section; this piece is probably not a complete chromosome. None of the chromosomes of this nucleus shows any indications of splitting. Another nucleus in the beginning metaphase contains 57 chromosomes in one section (fig. 2) and a small chromosome or piece of a chromosome in an adjoining section; here, also, there is no evidence that division of chromosomes has taken place. One daughter nucleus of an early anaphase comprises 50 chromosomes in one section and 4 complete chromosomes in an adjoining section (lower portions of figs. 8 and 9). Another daughter nucleus of an early anaphase comprises 54 chromosomes (upper portion of fig. 11) in one section and one chromosome or piece of a chromosome in an adjoining section. One daughter nucleus of a fairly late anaphase is apparently complete in one section and contains 56 chromosomes (fig. 15). This chromosome count is probably the most trustworthy. The tentative conclusion is that the diploid number of chromosomes is probably 56, but it may be as low as 54 or as high as 58.

In other urodeles the chromosome number (Wilson, '25) for the diploid group is usually 24, though in a few cases (e.g., *Amblystoma*) it is 28. Comparing the latter number with a probable 56 for *Cryptobranchus*, it is interesting to note the ratio and to speculate concerning its possible significance.

7. *Anomalies*

Only two cytological anomalies were encountered in the study of the cleavage nuclei. One is a case of multipolar mitosis and the other is a giant cell. Both were found in

the micromeres of a single egg in a very late blastula stage (stage 10). The case of multipolar mitosis is a tetraster in an early anaphase; each of the four chromosome groups appears to comprise the usual number of chromosomes and the entire cell is about twice the size of an ordinary micromere. The giant cell is about three times the diameter of an ordinary micromere, and its nucleus, which is in an early prophase, is of corresponding size. This nucleus is made up of several distinct vesicular structures, each consisting of several or many chromosomal vesicles in a compact group. Owing to the advanced stage of cleavage, it is not likely that these anomalies were due to polyspermy; it is more probable that they were caused by some injury to the egg.

DISCUSSION

The type of nuclear reconstruction involving the formation of chromosomal vesicles can no longer be considered rare. Chromosomal vesicles have been noted in a large number of species of animals belonging to several different phyla; some indication of the range of their occurrence may be gathered from the fact that they have been described in hydroids (Beckwith, '08; Hargitt, '09) and in Triton (Van der Stricht, '92; Braus, '95). In plants (Kater, '26 and '27) chromosomal vesicles have been described in only a few genera (bean, potato, tomato, onion). In animals chromosomal vesicles have been most frequently observed in the cleavage stages of ova, though they have been described also in the development and maturation of the germ cells; in plants they have been found in the growing root-tips of seedlings. Richards ('17) gives an extensive bibliography dealing with chromosomal vesicles, and the subject has been reviewed recently by Wilson ('25); hence it seems unnecessary to present here a comprehensive survey of the literature.

Interest in chromosomal vesicles has centered around the significance of these structures for the genetic continuity of individual chromosomes. Since this subject has been discussed by several authors, notably Richards ('17) and Wil-

son ('25), it need not be dwelt upon here. It is evident that, if the walls of the chromosomal vesicles persist during the resting stage, they serve to keep the metamorphosed chromosomes apart until the early prophases, when a new chromosome is formed from a portion of the material within each vesicle. In most cases where the formation of chromosomal vesicles has been observed, they do not all persist as separate entities beyond the telophase; in the resting stage more or less fusion of vesicles occurs, as in *Cyclops* (Rückert, '95), in *Crepidula* (Conklin, '01 and '02), and in *Cymbulina* (Nekrassoff, '09). In several cases, including *Macrobiotus* (Wenck, '14), *Fundulus* (Richards, '17), *Dinophilus* (Nachtsheim, '19), *Rana* (Swingle, '21), *Phaseolus* (Kater, '26), and *Cryptobranchus* as described in the present paper, chromosomal vesicles have been found in apparently undiminished numbers and with walls intact throughout the resting stage and the period of formation of new chromosomes; but in these forms a precise numerical correspondence with the chromosomes of the division phases has not been demonstrated. In *Lymnaea* (Crabb, '27) the maximum number of chromosomal vesicles found during the resting stage corresponds to the number of chromosomes in the division phases. In certain *Acrididae* (Eisentraut, '26) chromosomal vesicles are formed in a late prophase of the first maturation division of the spermatocyte, and the vesicular walls persist until spermatid formation. In *Ascaris megalocephala bivalens* the formation of two chromosomal vesicles representing the two chromosomes of the egg nucleus at the completion of maturation has been described and figured by Boveri (Wilson, '25, fig. 392A) as an anomaly, and this observation has been repeatedly verified by me in the course of routine laboratory work. In *Acrochismus*, S. H. Schrader ('24) found the egg nucleus or female pronucleus made up of eight chromosomal vesicles, corresponding in number to the chromosomes of the haploid group. In certain species of grasshoppers, McNabb ('28) found that during early cleavage the chromosomes assume a vesicular condition in the telophases and

retain their individual boundaries in all stages of interkinesis; the number of chromosomal vesicles is precisely the same as the number of chromosomes. This appears to be the only case of chromosomal vesicles occurring during cleavage in which such a numerical relation has been fully established.

It is apparent that, in several forms in which chromosomal vesicles have been intensively studied, fusion is either wholly absent or does not occur with enough frequency to impair the value of the material for the study of chromosomal history; and it is the chromosome, not the vesicular wall, whose transformations we must understand if we are to solve the entire problem of the genetic continuity of individual chromosomes. Since chromosomal vesicles do not occur in the nuclear cycle of all cells that divide to give rise to similar groups of chromosomes in successive cell generations, some mechanism other than vesicular walls must exist to insure the genetic continuity of chromosomes. It is probable that the metamorphoses of the chromosomes and the method of forming new chromosomes in the early prophase are fundamentally the same, regardless of whether each chromosome is inclosed in a vesicular wall, and some explanation must be sought for the presence of this wall other than as a device for insuring genetic continuity. From the viewpoint of chromosomal continuity, the vesicular walls are important mainly because in some cases they enable us to define, for purposes of study, the limits of individual metamorphosed chromosomes. Thus, chromosomal vesicles afford favorable material for the study of the metamorphoses of chromosomes, and this metamorphosis is important because it affords a natural analysis of the constitution of the chromosome, as well as an indication of the steps that must be retraced in the formation of a new chromosome out of a stem portion of the old. In the absence of vesicular walls separating individual chromosomes, the chromosomal history would probably be fundamentally the same, though temporary attachments by means of linin strands would be formed between adjacent chromosomes, e.g., as in *Tradescantia* (Sharp, '20).

The method of formation of the wall of a chromosomal vesicle described for *Cryptobranchus* in the present paper differs materially from that recorded by several authors working with other material. Conklin ('01 and '02) describes a chromosome of *Crepidula* as consisting of chromatin inclosed in a linin sheath; this sheath becomes the wall of the chromosomal vesicle. According to Richards ('17), a chromosome of *Fundulus* "consists of two substances, linin and chromatin, of which the former is in the nature of a sheath or sac, while the latter exists as a mass contained in the sheath"; a chromosomal vesicle begins its formation by the swelling of the chromosome and the breaking up of chromomeres into granules of chromatin which adhere to the inner surface of the sheath. Here, as in *Crepidula*, the sheath becomes the wall of the chromosomal vesicle. Substantially the same interpretation is reached by Kater ('26 and '27) in his studies of the chromosomal vesicles of *Phaseolus* and certain other plants. In *Cryptobranchus*, on the other hand, there are no indications of the existence of a chromosomal sheath at the time of the formation of the vesicles, and the wall of the vesicle is formed by a modification of the cytoplasm under the influence of the chromosome and a globule of karyoplasm.

In *Cryptobranchus*, as in *Fundulus* (Richards, '17), the new chromosomes formed in the chromosomal vesicles are set free by the dissolution of the vesicular walls. In *Phaseolus*, Kater ('26) found that "the chromosomal vesicles merely lose their achromatic content, contracting and giving rise to the new chromosomes, the old linin sheath apparently being continuous from one division to the next." This history Kater regards as very exceptional. The case of *Acrididae* described by Eisentraut ('26) is somewhat similar.

It is worthy of remark that, in the blastomeres of *Cryptobranchus*, nucleoli resembling those that occur during oogenesis (Smith, '12 a) are rarely found. Occasionally, one or two large smoothly rounded nucleoli, staining deeply with Bismarck brown, have been found during the early and middle prophases in nuclei of a late cleavage stage.

In the middle prophases of *Crepidula*, Conklin ('02) describes the extranuclear spindle fibers as directly continuous with the linin threads of the nucleus, which they closely resemble in every respect; like the linin, the spindle fibers branch and anastomose and are studded with oxychromatin granules. In *Cryptobranchus* there are no very definite granules in either the spindle fibers or the undifferentiated cytoplasm, though strands extending in the line of vision look like sharply defined granules. Even after the linin network has lost its chromatin granules and become very pale and attenuated, the linin network is distinguishable from the spindle fibers by its more delicate structure and by its manner of branching. The spindle fibers occasionally branch and anastomose, but almost always either directly or obliquely toward the equator of the spindle; the meshes of the linin reticulum are irregularly polygonal without a preponderance of strands extending in any particular direction. The linin network has been traced up to the point of its disappearance; before this takes place a few spindle fibers have invaded the vesicles, but I have been unable to demonstrate any continuity between spindle fibers and linin strands.

The question why chromosomal vesicles occur in some types of cells and not in others is ably discussed by Conklin ('02, pp. 45 and 46): "Such vesicles are found generally, if not universally, in the early divisions of ova, though they are not usually found in other mitoses. What is the cause of this difference? It occurs to me that it may be due to differences in the size and in the rapidity of division of blastomeres as compared with tissue cells." He cites his observations showing that chromosomal vesicles are proportional in size to the size of the cell (quantity of cytoplasm) in which they lie, and concludes that "in large cells where divisions succeed one another at short intervals the chromosomes begin the growth characteristic of the daughter nuclei, i.e., the absorption of substance from the cell body, before they have fused together, whereas in small cells or cells which divide only at long intervals the chromosomes fuse before the absorption of achromatic material begins."

It is evident that the formation of chromosomal vesicles is a device for the more rapid and effective performance, under stress of special circumstances, of work that the nucleus must accomplish during the resting stage. From the viewpoint of metabolic changes, the so-called resting stage is the most active of the entire nuclear cycle; it is the stage in which the nucleus is recovering from the losses sustained by division and is storing up substances that will later be discharged into the cytoplasm. All this requires interaction between nucleus and cytoplasm; the chromosomes must undergo structural changes in order that their surfaces of contact with surrounding fluids may be increased. As shown by the rotund appearance of the vesicles and their rapid increase in size from the moment of their formation, their walls are semipermeable; the vesicles expand by taking in fluid from the cytoplasm. In the resting stage the chromatin, mainly in the form of linin, is spread out in a close-meshed reticulum. Most of this network, with its basichromatin nodules, is located just within the vesicular wall; the formation of a vesicle for each individual chromosome increases the amount of surface to which the reticulum may be attached and facilitates its orderly distribution. During the resting stage a small amount of new basichromatin is formed, and during the early prophase the amount of new basichromatin is comparatively large. Only a small part of the total chromatin content of the vesicle is involved in the formation of the new chromosome; the remainder disintegrates and appears to be dissolved in the karyolymph. Thus, at the time of the disintegration of the vesicular walls, the fluid returned to the cytoplasm is quite different from the fluid that the vesicles absorbed; this fluid now contains the karyolymph and the products of visible materials that have been dissolved in it. It may be significant that chromosomal vesicles are characteristic of an early stage of development, when the inheritance that is somehow compressed within the germ is just coming to expression.

SUMMARY

1. The number of chromosomes in the cleavage nuclei of *Cryptobranchus* has not been determined beyond question, but is probably fifty-six.

2. The chromosomes are elongated structures of fairly uniform thickness, but of various lengths. During the late anaphases the shortest chromosomes are all rod-shaped, those of intermediate sizes are rod-shaped or hook-shaped, while the longest ones are V-shaped or U-shaped. These various kinds of chromosomes are quite regularly distributed in characteristic patterns during the metaphase, anaphases, and telophases.

3. During the early telophases each chromosome becomes inclosed in an individual sac or vesicle which together with its contents is called a chromosomal vesicle. The vesicular membrane is of cytoplasmic origin, but is developed under the influence of the chromosome and a droplet of karyolymph which is formed between the chromosome and the surrounding cytoplasm.

4. A precise numerical correspondence between chromosomes and chromosomal vesicles has not been established, but it is evident that most, if not all, of the chromosomal vesicles retain their individuality during the resting stage and early prophase. Degeneration of the vesicular walls is not apparent until after the new chromosomes have been fully formed.

5. There is no nuclear membrane other than the walls of the individual chromosomal vesicles.

6. The transformation of the telophase chromosome into the reticulum of the resting stage and the manner in which a new chromosome is formed from a portion of this reticulum are described in detail.

7. In the early prophases each developing chromosome is embedded in a sheath or matrix of less deeply basophilic material, which disappears before the middle prophase is reached. The existence of a chromonema in the completed chromosome is problematical.

8. During the very late telophases, resting stage, and particularly in the early prophase, many basichromatin nodules become detached from the reticular framework and undergo degeneration and final absorption in the karyolymph. During the middle prophase the portion of the reticulum that does not take part in the formation of the new chromosome also degenerates and is absorbed. At the close of the middle prophase, the modified karyolymph is set free by the disintegration of the vesicular wall and allowed to mingle with the cytoplasm.

9. The formation of chromosomal vesicles is interpreted as a device for doing more rapidly and effectively, under stress of special circumstances, the work that the nucleus must accomplish during the so-called resting stage.

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PLATE 1

EXPLANATION OF FIGURES

1 Polar view of the chromosomes of a nucleus just entering the metaphase. The arrangement of the chromosomes is more characteristic of a late prophase, equatorial view. The section is 13μ thick and contains all the chromosomes of this nucleus, excepting one small piece in an adjoining section. $\times 750$. Stage 8.

2 Polar view of the chromosomes in an early metaphase. The section is 25μ thick and contains all the chromosomes of this nucleus, excepting one short chromosome or piece of a chromosome in an adjoining section. $\times 750$. Stage 7.

3 Early metaphase, equatorial view. Most, if not all, of the chromosomes are splitting. The section is 13μ thick. $\times 750$. Stage 6.

4 Early metaphase, oblique view. Most of the chromosomes are splitting. The section is 8μ thick. $\times 750$. Stage 8.

5 Late metaphase, equatorial view. Only a small part of the nucleus is present in this section, which is 9μ thick. $\times 750$. Stage 8.

6 and 7 Equatorial views of a late metaphase as seen in two successive sections, each 10μ thick. $\times 750$. Stage 8.

8 and 9 Equatorial views of an early anaphase as seen in two successive sections, each 12μ thick. The chromosome group represented in the lower parts of these figures is complete in these two sections. $\times 750$. Stage 6.

10 Early anaphase, equatorial view. 8μ thick. $\times 750$. Stage 4.

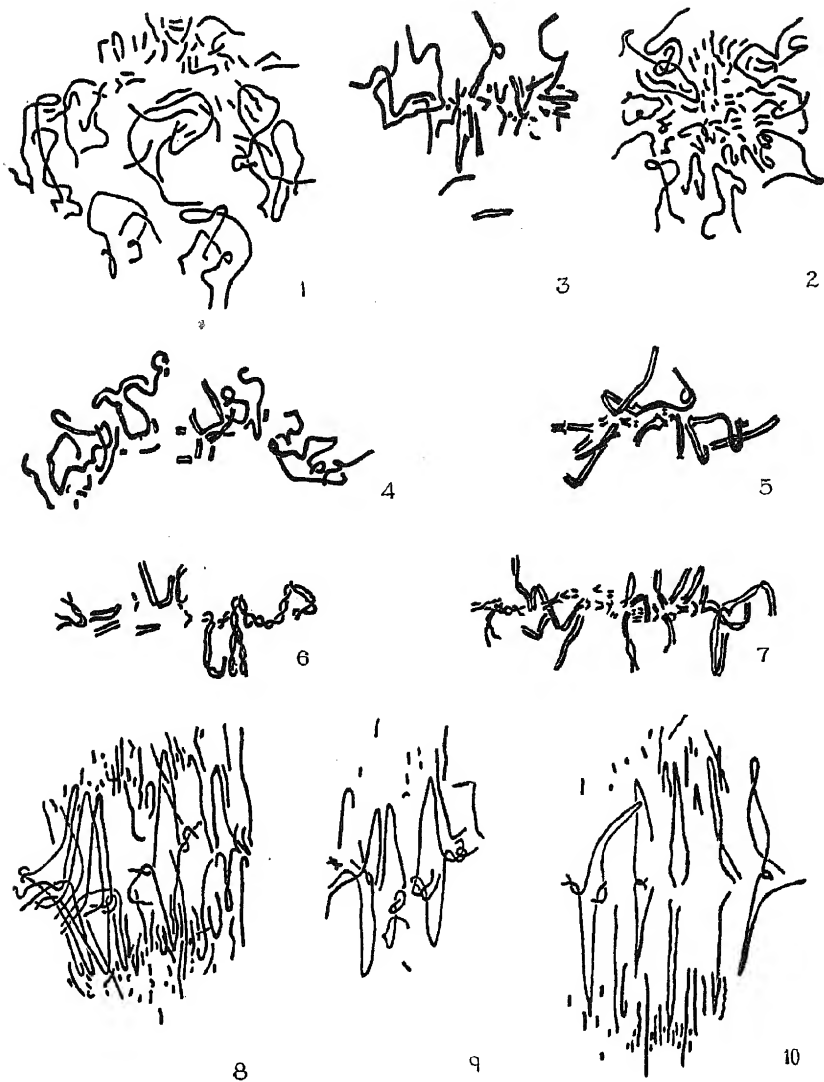


PLATE 2

EXPLANATION OF FIGURES

11 Early anaphase, equatorial view. All the chromosomes of the daughter group represented in the upper part of the figure appear to be confined to this section, with the exception of one short chromosome found in the preceding section. The section is 12μ thick. $\times 750$. Stage 6.

12 Middle anaphase, equatorial view. 12μ . $\times 750$. Stage 6.

13 Late anaphase, equatorial view. 12μ . $\times 750$. Stage 6.

14 Late anaphase, equatorial view. 10μ . $\times 750$. Stage 8. In this and the following figures only a single daughter nucleus is shown.

15 Late anaphase, equatorial view. The section is 25μ thick and none of the chromosomes of this daughter nucleus is found in adjoining sections. $\times 750$. Stage 7.

16 Late anaphase, equatorial view. 8μ . $\times 750$. Stage 6.

17 Beginning telophase, equatorial view. 13μ . $\times 750$. Stage 6.

18 and 19 Equatorial views of a beginning telophase as seen in two successive sections, each 8μ thick. $\times 750$. Stage 4.

20 and 21 Equatorial views of an early telophase as seen in two successive sections, each 10μ thick. $\times 750$. Stage 8.

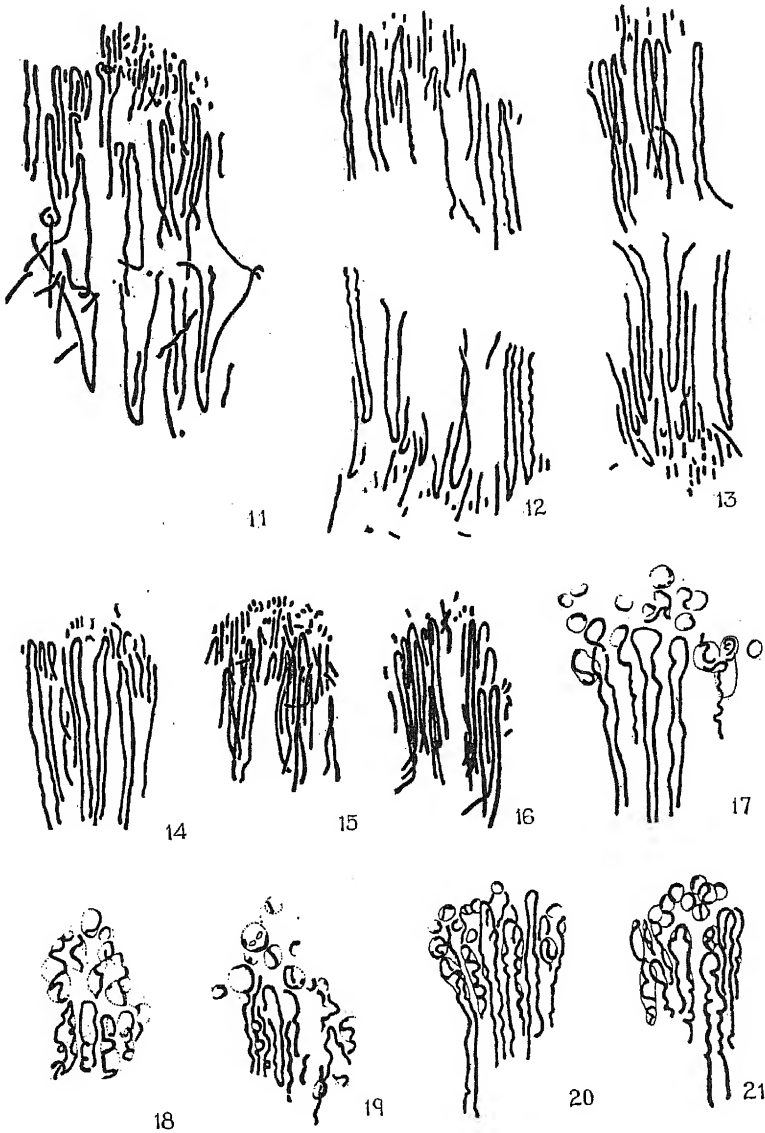


PLATE 3

EXPLANATION OF FIGURES

- 22 Early telophase, equatorial view. 15 μ . \times 750. Stage 6.
- 23 Early telophase, equatorial view. 13 μ . \times 750. Stage 6.
- 24 Middle telophase, equatorial view. 10 μ . \times 750. Stage 6.
- 25 Middle telophase, equatorial view. 12 μ . \times 750. Stage 6.
- 26 Middle telophase, equatorial view. 13 μ . \times 750. Stage 6.
- 27 Middle telophase, equatorial view. 13 μ . \times 750. Stage 6.
- 28 Middle telophase, oblique view. 8 μ . \times 750. Stage 5.
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- 32 Middle telophase, polar view. 13 μ . \times 750. Stage 3.
- 33 Middle telophase, polar view. 13 μ . \times 750. Stage 3.

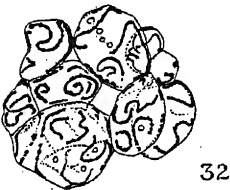
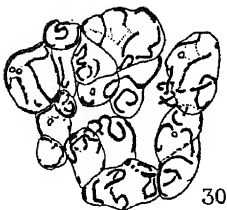
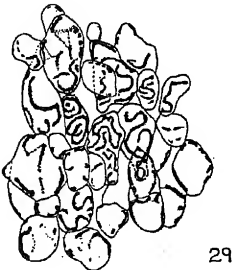
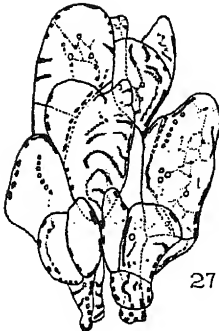
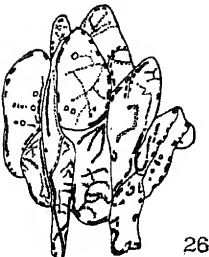
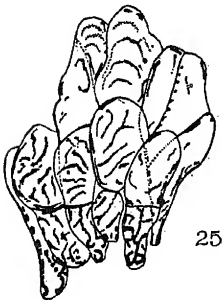
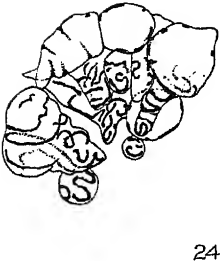
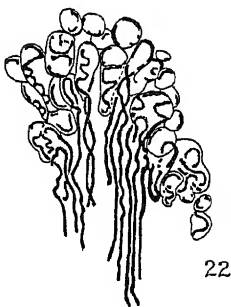


PLATE 4

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- 42 Early resting stage, equatorial view. $9\ \mu$. $\times 750$. Stage 6.
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- 45 Beginning prophase, equatorial view. $8\ \mu$. $\times 750$. Stage 8.
- 46 Very early prophase, equatorial view. $9\ \mu$. $\times 750$. Stage 8.
- 47 Tangential section through a single chromosomal vesicle, very early prophase. $6\ \mu$. $\times 2250$. Compare this figure with figure 41.

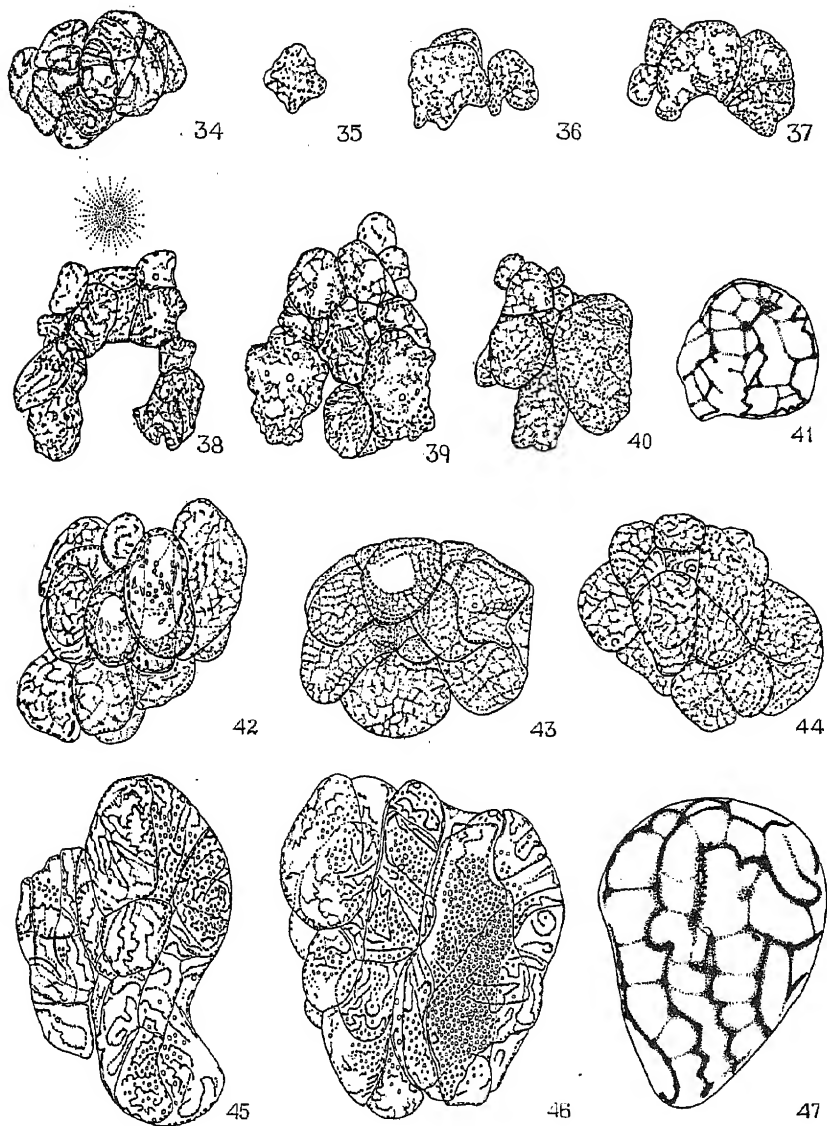


PLATE 5

EXPLANATION OF FIGURES

48, A to E Portions of chromosomes in early prophase, drawn to illustrate successive developmental stages. Each chromosome is from a different nucleus, save that each transverse section (lower portions of B to E) is taken from the same nucleus as the accompanying side view (B to E above). $\times 3750$.

49 Early prophase, equatorial view. 9μ . $\times 750$. Stage 8.

50 Early prophase, equatorial view. 10μ . $\times 750$. Stage 6.

51 Middle prophase, equatorial view. 11μ . $\times 750$. Stage 6.

52 Middle prophase, equatorial view. 10μ . $\times 750$. Stage 8.

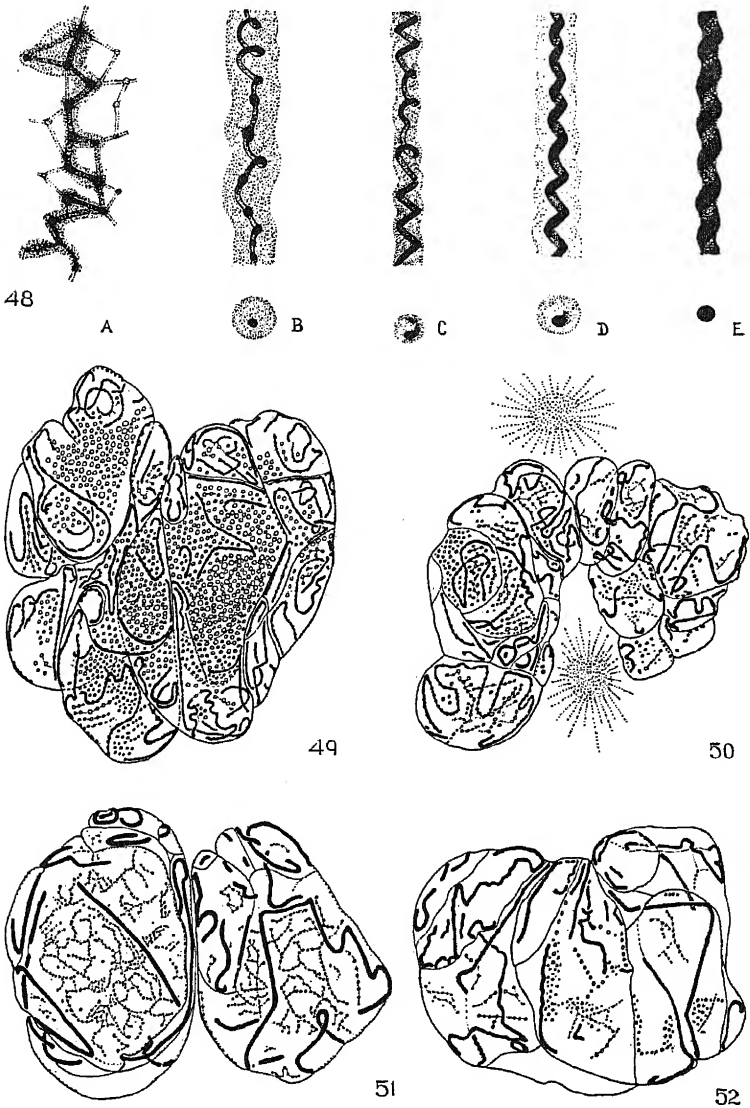
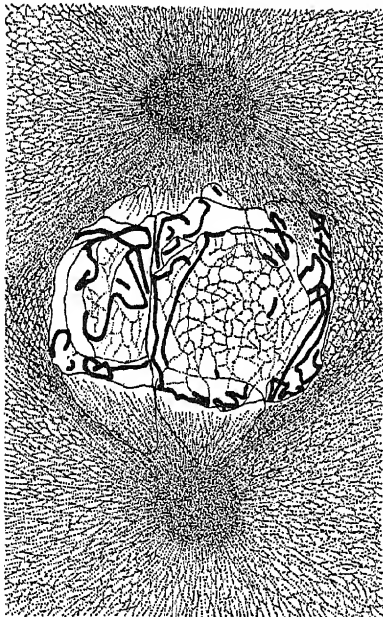


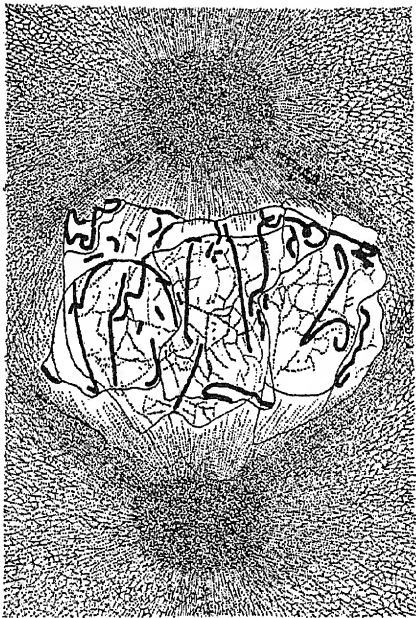
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- 58 Late prophase, equatorial view. $11\ \mu$. $\times 750$. Stage 6.



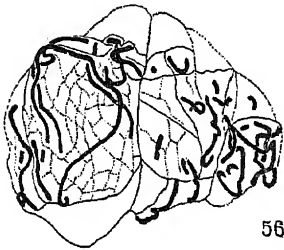
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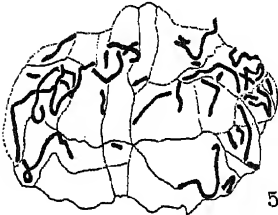
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ON THE DEVELOPMENT OF IMAGINAL BUDS IN NORMAL AND MUTANT DROSOPHILA MELANOGASTER

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TWO TEXT FIGURES, FOUR CHARTS, AND SIX PLATES (SIXTY-FOUR FIGURES)

AUTHOR'S ABSTRACT

Definite information concerning the time of development and location of the different imaginal discs of *Drosophila melanogaster* was needed in order to interpret especially the gynandromorphs, mosaics, and intersexes that have been extensively reported in cultures of this fly. This information was also desirable for many of the mutant types. It was not known, for example, when an organ was reduced or absent, whether its imaginal disc showed a corresponding reduction, or whether it was full size in the larvae and pupae, and failed to carry through to the later stages.

Three mutant types with eyes smaller than those of the wild type, namely, lozenge, bar, and eyeless, were examined. It was found that there is a corresponding difference in size as far back as the imaginal disc could be detected. Similarly for the two mutants, vestigial and no-wing. Conversely for the mutant, bithorax, in which the metathorax is larger than the normal and has assumed many of the characters of the normal mesothorax, the imaginal disc was correspondingly enlarged.

It follows that the effects of the mutant genes for these characters can be observed in the very earliest condition of the imaginal disc.

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INTRODUCTION

The breeding work in *Drosophila melanogaster* in the Columbia Laboratory has led to the discovery of a large number of mutant types which show modifications from the wild type either in the function or in the structure of the body. Physiologically, there are mutant types of different degrees of sterility and other types having lethal effects on different stages of development. Morphologically, there are mutant types with a structure or an organ changed or lost in all gradations.

Detailed information concerning the development of the imaginal discs of *Drosophila melanogaster* is needed for several purposes. Comparative information of other species does not suffice when certain specific problems in this species are considered. For example, it was unknown whether, when an organ is rudimentary, such as the wing of vestigial, the imaginal disc is also absent or reduced, or, if full-sized, whether only a part of the disc forms the rudiment. It was also important to determine at what stage in the development of the discs of mutant rudimentary types the earliest indication of the change in character can be seen. This may be especially significant when embryological questions arise. Again, the interpretation of gynandromorphs, mosaics, and intersexes often depends on an exact knowledge of the relation of the imaginal discs to the parts of the body that arise from them.

With these objectives in view, the present work was started. Since a study of the developmental stages of mutant characters presupposes a working knowledge of their normal development, a study of the development of the wild type has been carried through with special reference to the time rela-

tion of the appearance of different imaginal buds and their critical phases of development into definitive structures. With this information at hand, seven different mutant characters were then studied and comparisons between them and the mutant types were made.

The account here is limited to the study of three imaginal discs of the wild type: the eyes, wings, and halteres. Other imaginal discs are mentioned whenever necessary. The mutant types which have been studied are three characters involving eye size, viz., lozenge, Bar, and eyeless; two involving wing size, viz., vestigial and no-wing; one involving the halteres, viz., bithorax; and one involving a lethal effect on early pupa, viz., 'giant larva.'

ACKNOWLEDGMENT

The author is indebted to Prof. T. H. Morgan for supervising the work and Dr. A. H. Sturtevant for suggestions, to the Rockefeller Foundation for the appointment to a Fellowship in 1925-27, and Yenching University, Peking, China, for a grant from the Research Fund.

MATERIAL AND METHOD

The Florida strain of *Drosophila melanogaster* furnished the material for the study of development of 'wild type.' This strain, inbred for a number of years, shows relative genetic purity. Before the work was started, sister and brother matings were made, and inbreeding was carried on for three or four generations. Again, selections were made of pairs that gave the largest and healthiest families in each generation. All the mutant types used are from the Columbia Laboratory and have known genetic constitution. Most of the mutant types are homozygous stocks; a few are balanced stocks. In the latter case proper matings were made to secure suitable material. The genetic purity of the mutant types was secured in the same way as described for the wild type.

In order to determine the conditions of mutant characters in their developmental stages, it is necessary to use those which differ from the wild type in a very striking manner. This requirement was fulfilled by choosing mutants having one entire organ missing, such as eyeless, or an extra portion of the body substituted, such as bithorax, or a structure widely different from that of wild type, like vestigial wing.

Care was taken to secure uniform and favorable culture conditions. The bananas used were always dead ripe, but never overcooked. Forty cubic centimeters of food were put in each half-pint milk bottle and one drop of a yeast suspension, containing one Fleischmann's yeast cake in 100 cc. of water, was added after the food had set. Since no paper was used in the bottles, the larvae on reaching maturity crawled up the sides of the bottles and formed puparia on the glass, and they were easily removed with a fine flexible scalpel.

Flies were raised from eggs to the adult stage in a constant temperature of 25°C.

The rate of growth of *Drosophila* is greatly influenced by temperature and by the food conditions to which the culture is subjected. The age of the different stages must be known in order to determine accurately the time of appearance of certain structures or organs during development. The time factor is again a matter of importance when comparing certain structures or organs of mutant types with those of the wild type in different stages of development. The time factor is controlled by obtaining eggs freshly laid, that is, within one hour. On a strip of glass that fits into the vials is placed a piece of green blotting-paper thoroughly soaked in a yeast culture. Then a thin layer of cooked banana-agar food is spread on the top of the paper. A female *Drosophila* lays very few eggs the first three days. On the fourth and several succeeding days, she lays about one hundred eggs a day. To insure getting a large number of eggs and eggs of the same age, the ten or more females used were well fed first. They were not less than four nor more than eight days old. One

hour later, the females were removed and the vial containing the eggs was put into an incubator at 25°C. About twenty-four hours later, nearly all the eggs had hatched or were about to hatch. Young larvae not more than one hour old were carefully removed from the food with a camel's-hair brush and put into the culture bottles. To eliminate a crowding effect, the number of young larvae put into each bottle was limited to not more than sixty. The larval period of *Drosophila melanogaster* covers four days and the pupal period another four days, under the temperature and food conditions used. The material for studying the development of larvae was obtained from samples of larvae taken at eight-hour intervals throughout the larval period. Larvae which had ceased feeding and remained quiet on the side of the bottle were in the last stage and called mature larvae. Approximately two hours later, their anterior spiracles were everted and the larvae have entered the prepupal period which covers a little over eleven hours at 25°C. Important events take place in the middle and at the end of the prepupal period. Samples of prepupae were taken every hour until the end of that period. The prepupal period is judged to be complete when the head and thorax and their appendages have just been everted. For the pupal period samples of pupae were taken every four to eight hours.

Among the fixatives tried, Gilson-Carnoy proved to be the most practical. Owing to its rapid penetrating power, total larvae were fixed in this fluid almost instantaneously, leaving no shrinkage nor distortion of shape or size of the larvae. As larvae of different stages have to be carefully measured, any gross postmortem change in shape or size is eliminated. Pupae were also fixed in this fluid. In order to insure the penetration of the fluid through the very tough pupal skin, a puncture was often made on the dorsal side of the pupa. The time of fixation varies with the size and texture of the material, from ten minutes for young larvae to several hours for old pupae. Materials for cellular structures were prepared by the usual histological technique. It is possible to

obtain good serial sections of whole larvae or pupae with a very sharp knife if the materials are embedded in hard paraffin. The tough larval skin and brittle pupa case offer serious difficulties. All the primordia used for comparison of mutant types with wild types were dissected out under a binocular microscope and mounted in toto. Dissection can be carried out successfully with a little patience and practice. The advantage of this procedure is its accuracy and its saving of time. The primordia dissected out in toto show all the important features. They can be fairly accurately measured and compared without the laborious method of reconstruction necessary in case of serial sections. Further details as to methods are given in the descriptions of the individual cases.

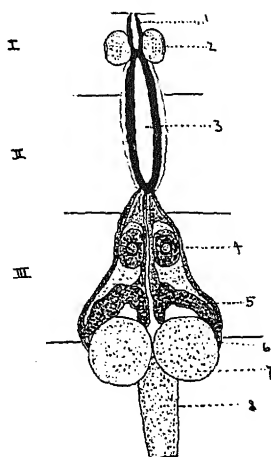
THE CEPHALIC COMPLEX OF WILD TYPE

In *Drosophila*, as in other Diptera, most of the prospective organs of the adult are laid down in the larva in the form of primordia—Weismann's 'Imaginal discs' or buds. The head of an adult *Drosophila* is represented at the end of the larval period (the mature larva) by two pairs of primordia (text fig. A and chart 1), a large pair and a small pair. The pair of large primordia is conveniently termed the cephalic complex and the pair of small ones, the labial buds. The latter are located on each side of, and close to, the external surface of the base of the oval hooks in front of the cephalopharynx. They suggest the appearance of two balls with the outer free surface a little compressed. On the compressed surface of each bud a narrow slit runs vertically from the center toward the margin of the bud.

The large cephalic complexes extend from the posterior extremity of the cephalopharynx to the anterior portion of the larval ganglia (text fig. A). They are subtriangular in shape, with the apices joined anteriorly and with a broad base posteriorly. Their short edges meet below the mid-dorsal line of the larva and above a large blood sinus, in which the oesophagus lies, and into which the dorsal vessel opens through a ridge known as 'Weismann's bridge.' Each

consists posteriorly of a large optic bud which gives rise to the compound eye, and a small antennal bud. Anterior to the latter is a narrow stalk-like portion forming the apex of the whole complex. This portion has been described by Lowne ('92) in the blowfly, *Calliphora*, as the prefacial bud. Its exact nature is not very clear in the case of *Drosophila*.

The optic bud is somewhat like a shallow cup with its concave surface fitting over the larval ganglia on their dorsal and anterior surfaces. An optic stalk serves as the connec-



Text fig. A Diagrammatic longitudinal section through the primordia of the cephalic complex in the mature larva. *I*, *II*, *III*, pro-, meso-, and metathoracic segments of larva; 1, the oral hooks; 2, labial buds; 3, larval pharynx; 4, antennal buds; 5, optic buds; 6, optic stalk; 7, larval ganglia; 8, larval nerve cord.

tion between the bud and the ganglia on the outer surface. The margin of the bud, except the point at which the optic stalk arises, is slightly thickened in the form of a rim, leaving a thinner central portion. The margin of the bud is in continuation with the membranous portion of the cephalic complex, covering most of the outer surface of the latter.

The cellular structure of the whole complex is of rather simple type. The cells are known to be 'epiblast,' uniform in size and shape, but very much smaller than other cells in the larval tissues. They have large nuclei slightly irregular

in outline. The cells in the periphery of the complex seem to be a little elongated and columnar, while those in the interior are cubical in shape. The cells of the optic bud in the mature larva have already exhibited the beginning of differentiation. On the inner surface of the bud, the cells have a definite arrangement. There are four terminal cells and six basal cells arranged around a deeply staining axis. These form a cylindrical unit. Krafka ('24) first pointed out that these units are the rudiments of the ommatidia. Under proper illumination and magnification they can be recognized in specimens stained in toto.

CHART 1
Primordia of the head

Primordia in mature larva	Labial buds	Cephalic complex	
		Antennal buds	Optic buds
Definitive organs in adult	Proboscis	Antennae	Compound eyes
	Lower part of the head	Front part of the head	Dorsal part of the head

The optic buds in mature larvae

The optic buds in mature normal larvae were taken as a measure for comparison with those of eye mutants, such as Bar, lozenge, and eyeless. At this stage, the optic buds have attained their final shape and size. The buds from larvae of earlier stages showed a wider range of variation and are therefore unsuitable for exact measurement. The mature larvae form prepuparia within one hour. During the first three hours of the prepupal period, practically no increase in size nor change in shape of the optic buds can be observed. However, the thickness of the bud is slightly increased without affecting its general size. This is to be expected, since, during the formation of the puparium by the shortening of the larva, the optic buds and other buds are brought very near each other, almost in a compact mass. The optic buds from a prepupa one hour old were measured to check up

those from mature larvae. They were dissected out and the length and width of the buds measured with an ocular micrometer in a calibrated compound microscope, and the area of the whole bud calculated in terms of square micra. The mature larvae and the one-hour prepupae were also measured with the ocular micrometer under a binocular microscope in terms of square millimeters in area. Their measurements are recorded in table 1. The mean size of the mature larva was 4.65 mm. \pm .062 for the male and 5.28 mm. \pm .069 for the

TABLE 1
Optic buds of wild type

WILD TYPE	SEX	NUMBER OF CASES	MEAN	S.D.	P.E. OF MEAN
Length (size) of larva					
Mature larva	♂	30	4.65	\pm .50	\pm .062
	♀	30	5.28	\pm .56	\pm .069
One-hour prepupa	♂	25	2.31	\pm .17	\pm .020
	♀	29	2.59	\pm .20	\pm .025
Optic buds (area)					
Mature larva	♂	46	535	\pm 102	\pm 10.14
	♀	51	618	\pm 114	\pm 10.53
One-hour prepupa	♂	36	458	\pm 93	\pm 6.76
	♀	40	515	\pm 122	\pm 12.99

female; and that of one-hour prepupa, 2.31 mm. \pm .020 for the male and 2.59 mm. \pm .025 for the female. The mean size of optic buds was 535 \pm 10.14 for the male larva and 618 \pm 10.53 for the female larva, and that of one-hour prepupa was 458 \pm 6.76 for the male and 515 \pm 12.99 for the female.

The measurement of males and their optic buds was made because, in the stock of the eye-mutant lozenge that was used, only males show the character. As described above, the optic buds have the shape of a shallow cup. The more or less uneven contour in the convex surface of the bud makes it

difficult to measure its thickness. The size of the bud, obtained by multiplying its length by its width, in terms of area, is therefore to be considered only approximate, but it is consistent in all the cases studied. The rather delicate operation of dissecting out the optic buds from larva and pupa made it difficult to get as large a number of buds as one would wish. The measurement of the buds, based on a rather small number of cases, proved on statistical treatment to be sufficient for the purpose.

Development of the cephalic complex

The origin of the cephalic complex, its site of invagination, and its mode of formation do not seem to be the same in all Diptera. The latest information on this subject is given in a paper by Snodgrass ('24). It is unnecessary here to go into all the morphological complications and contradictions of opinions on the subject. However, one example may be cited. According to Snodgrass, in the apple maggot, *Rhagoletis*, the anterior part of the pharyngeal roof is convex and covers an anterior section of the pharyngeal cavity called the atrium. From the posterior end of the atrium the roof of the pharynx is produced upward as a dorsal pouch. This pouch soon divides into two wings, which are produced backward nearly as far as the end of the pharynx, and are then prolonged into two long, stalked sacs. The stalked sacs, continuing backward from the ends of the pouches, are the frontal sacs which later contain the buds of the imaginal antennae and compound eyes. In *Drosophila melanogaster* the head complex has been traced back to the frontal-sac stage. Its earlier development requires further study before a definite conclusion can be reached. The difficulty of determining this point arises from the minute size of the young larva and its rapid development. Moreover, my attention was directed rather to the mode of development of the bud from the frontal-sac stage onward to its final shape than to its earlier phase of development. Since most of the imaginal buds found in *Drosophila* show a very close similarity in

structure and location to those in the apple maggot, it may perhaps be inferred that the mode of origin of the cephalic complex is essentially similar in these two forms.

In *Drosophila* the young larvae that have just hatched, up to the end of the first eight hours, show no signs of primordia. In other words, all the primordia are formed in the early stages of the larval period and none in the embryo stage. During the next eight hours or at the end of sixteen hours, the primordia of the cephalic complex in its simplest condition can be identified for the first time as a symmetrical pair projecting backward side by side from the posterior extremity of the larval pharynx and above the oesophagus. They appear to be in the form of two sacs, called frontal sacs (fig. 1). Their anterior end, in contact with the pharynx, is elongated like a string, increasing gradually in size toward the posterior free end. A narrow groove runs longitudinally along the middle line of each sac. The sac appears to be made up wholly of very minute 'epiblast' cells, more or less uniform in size and shape. The cells have rather large nuclei filling up a great part of each cell. Among the cells there are a few that are about twice as large as the small ones. They are in mitotic division. The mode of origin of these frontal sacs has not been conclusively shown. Apparently they come from the dorsal pouch, which in turn arises from the atrium, as in the apple maggot. In the next eight-hour period, sixteen to twenty-four hours, there is little change, except a slight increase in size.

At the end of thirty-two hours (figs. 2 and 4), the anterior narrow portion of the sac has become very much elongated and the posterior free portion much enlarged and somewhat oval in shape. The narrow groove, beside being elongated, has become broadened at its posterior end. At this stage, the posterior ends of the sacs are still free in the mesothorax of the larva. By the end of forty hours, considerable change in the shape of the sacs has taken place (figs. 3 and 5). The narrow anterior portion has increased more in width than in length, especially in the middle region. A convex surface,

due to outpushing, begins to be noticed. The posterior portion has greatly increased in size and become nearly circular in shape with a little protrusion at the posterior end. From this protrusion the optic stalk is formed. The stalk at this stage is not definitely connected with the larval ganglia and is composed of only one kind of large cells. No nerve fibers as yet can be observed. A zone of large cells in active mitotic division marks off the anterior from the posterior portion of the sac. At this stage, the frontal sac is no longer a simple sac; even though the regions have not been definitely marked off, the beginning of the separation of one from the other has already started. A frontal sac of this kind may be well called an incipient cephalic complex.

At the end of forty-eight hours (fig. 6), the cephalic complex can be definitely identified as such, with two distinct portions, an anterior and a posterior one. The former is the antennal bud and the latter, the optic bud. The antennal bud is in the form of a ring, somewhat compressed on the dorsal and ventral sides. The optic bud, occupying the whole posterior portion of the cephalic complex, is circular in shape, the outer surface of it being convex with a thicker rim on the periphery, except the point at which the optic stalk arises. The cephalic complex, as a whole, is a more or less triangular sac with a thin membrane forming the outer wall and the antennal and optic buds the inner wall. There is a thin region of epiblast between these two buds, and there are two bands of epiblast, forming the two arms of the complex, that meet at the apex. This is the stage at which the antennal and optic buds are distinctly separated and thus is the cephalic complex definitely laid down. In the following periods the changes taking place in the cephalic complex seem to be due to an increase in the size of the whole or of certain regions of the complex.

During the forty-eight-to-fifty-eight-hour period (fig. 7) the whole complex has continued to increase in size, especially on the ventral and external borders of the optic bud, and slightly in the region around and anterior to the antennal

bud. The general outline of the cephalic complex as a whole has not been altered at all; the two buds are more distinctly separated than in the previous stage. The increase in size of the whole cephalic complex continues from fifty-six hours to about ninety to ninety-six hours—the end of the larval period (figs. 8, 9, 10, 15). During that period the optic bud becomes more concave on the inner surface, finally assuming the shape of a shallow cup. The dorsal and outer borders of the bud become pronouncedly thicker as development advances. The antennal bud appears to be segmented at the end of sixty-four hours (fig. 8), that is, a smaller segment has separated slightly from the original large bud. The region around and anterior to the antennal bud becomes more extended in area in the period following.

The cephalic complex as a whole seems to have attained its definite size and form at the end of eighty hours or a little later (fig. 10). At this stage, the optic buds have become fused with the larval ganglia on their anterodorsal aspect. This connection is of a membranous character, and the buds and the ganglia may be readily separated by dissection. During the last stage of the larval period the growth process seems to consist in an increase in thickness of the complex. The cephalic complexes from the mature larvae do not appear to be appreciably larger than those of the seventy-two-to-eighty-hour-old larvae, but they do appear to be different from the latter in being thicker and more compact and darker in stained preparations. The cells in the optic buds are already arranged in definite units, which are the rudiments of the ommatidia.

When the larva reaches the last stage of development, it ceases feeding, crawls out of the food and up the sides of the bottle, and, approximately two hours later, it becomes quiescent and finally everts its anterior spiracles. The larval skin gradually shortens, loses all signs of segmentation, and becomes the puparium. This shortening is seen more conspicuously in the few anterior segments than in those at the posterior end. The retraction of the anterior segments brings

all the imaginal buds located in them into their final position in the puparium before they are everted as the definitive organs of the adult. During the first four hours they remain as they were when the puparium was formed. The movement of the imaginal buds takes place in two steps, a preliminary one at the end of five hours and a final one at the end of eleven hours at 25°C. During the preliminary movement the wing and halter buds have been formed and remain attached to the original thoracic buds. The leg buds also are partially everted. In the final movement all the imaginal buds are completely everted; thus the head, thorax, and abdomen are all visible in dorsal view. The outline of the compound eyes, wings, and legs are definitely fixed and the topographical relations of these organs to one another and to the regions of the body are finally determined. The pupal period covers about four days. During this period the differentiation into finer details of the integrating parts of different structures takes place.

THE EYE-MUTANT CHARACTERS

In the following section the development of certain eye-mutant characters is described, viz., lozenge-3, Bar, and eyeless-4.

Lozenge-3 (lz^3)

The gene for lozenge lies in the first chromosome at 27.7 units. The compound eyes of lozenge are smaller than the wild type, translucent, deep brownish red in color, and abnormal on the surface, since the facets run together into a smooth, almost hairless sheet, often suggesting the presence of moisture or glaze. The lozenge female is sterile. The character is maintained in a balanced stock. The lozenge male is mated to 'double-X' yellow female. Two classes of offspring are obtained in the F_1 , lozenge males and 'double-X' yellow females.

In very young lozenge male pupae immediately after pupation (fig. 12), the eyes are proportionately smaller than those

of the wild type (fig. 11) of the same size and corresponding age. The size relations found in the pupa are also present in the larval stage. In the mature lozenge male larvae the optic buds (fig. 16) were found to be smaller in size than those of the wild type (fig. 15) of the same age and size. The cephalic complex of lozenge larvae appears to be similar to that of wild type in respect to its shape and structure. The region in front of the optic bud and the region which contains the antennal bud and the anterior apex of the complex are approximately of the same size in both cases. The only difference that could be detected between these two types of

TABLE 2
Optic buds of lozenge-3

LOZENGE	SEX	NUMBER OF CASES	MEAN	S.D.	P.E. OF MEAN
Size					
Mature larva	♂	30	4.473	$\pm .37$	$\pm .046$
One-hour prepupa	♂	20	2.276	$\pm .14$	$\pm .021$
Optic buds					
Mature larva	♂	54	360	± 57	± 5.30
One-hour prepupa	♂	38	312	± 44	± 4.82

cephalic complexes is in the size of the optic bud, and this difference is conspicuously shown. The optic buds were measured with an ocular micrometer under a compound microscope, and the area of the bud calculated in terms of square micra. The curvature of the convex surface and the thickness of the bud are difficult to measure. The measurements obtained are therefore only approximate. The mean size is 360 ± 5.30 (table 2). The optic bud of wild type has the mean 535 ± 10.14 at the same age. But the difference between the size of the lozenge larvae and wild-type larvae is insignificant (table 2). In spite of the similarity in size of these larvae, the optic buds in the lozenge mutant are 33 per cent smaller than those of the wild type.

In the first few hours of the prepupal period the imaginal buds show very little or no increase in size or change in shape. Lozenge male prepupae were measured, and the cephalic complexes, including optic buds, were dissected out and measured. As shown in table 2, the optic buds have the mean 312 ± 4.82 , while the optic buds from wild type of same age have 458 ± 6.761 (table 1). The size of the prepupae of both types proved to be about the same (lozenge, $2.276 \pm .0211$; wild type, $2.31 \pm .020$). At this stage, the size of the optic bud is slightly smaller than in the previous stage, due to the compressing effect alluded to before. The difference in the optic buds of these two types remains constant, lozenge being again 32 per cent smaller than wild type. This second value checks perfectly with the first one, indicating that the optic buds from which the first value was obtained were from larvae of the same age and stage of development—which rules out the possibility that the small optic buds from lozenge larvae might be in an earlier stage of development or from younger larvae. An attempt was made to trace the effects of lozenge into earlier stages of development, but the data are insufficient to make any positive statement.

A mutant called 'glaze' in *Drosophila virilis* is regarded as parallel to lozenge in *Drosophila melanogaster*. They show genetic similarity, but their morphological identity has not been shown. Johanssen ('24) found that the ommatidia of the adult fly are shorter than those of the normal eye; the rhabdomeres are strongly developed, the postretinal fibers and the ganglionic plate are coarse and well marked; the facets are somewhat irregular in outline; the pigment of the ommatidium is more or less dense basally and apically. It is further pointed out by him that 'glaze' resembles a late pupal eye in its proportion and its distinct postretinal fibers, but differs in the strongly developed rhabdomeres.

Bar (B)

Bar (57 units) is a dominant sex-linked character. The eye is restricted to a narrow vertical strip. The number of

facets in Bar is very much reduced. There are $70 \pm$ in the male, $80 \pm$ in the female; whereas in wild type there are 740 in the male and 780 in the female. The female, heterozygous for Bar, has an eye that is intermediate between the rounded eye of the wild type and the narrow band of Bar stock ($360 \pm$ facets).

In the Bar-eyed fly the size of the head is conspicuously smaller than in the wild type, due to the reduced size of the compound eye. In very young pupae immediately after pupation, the head of Bar-eyed (fig. 13) individuals appears to be distinctly smaller than that of wild type (fig. 11), even although at this stage the facets have not yet developed. It is apparent that in this stage the area for the eye has been fixed and the material for the development of eye elements definitely laid down. This fact was first brought to light by Krafka in 1924. It was shown by him that the ommatidia of Bar eye show no structural difference of any sort from those of wild type. The optic tract is smaller.

Full-grown larvae of Bar-eyed stock were obtained from cultures raised from eggs laid within one hour and with environmental conditions carefully controlled. The larvae were measured, and the cephalic complexes, including the optic buds, were dissected out, their length and width measured, and the area of the optic buds calculated. The cephalic complex appears to be similar in both Bar and wild-type larvae in respect to shape and structure. As to size, the optic buds from Bar larvae (fig. 17) are distinctly smaller than those of wild type (fig. 15), but the region anterior to the optic buds, including the antennal buds and the apex of the complex, is approximately the same. This fact indicates clearly that the effect of the gene is localized in the optic bud. The Bar-eye condition in the optic buds is approximately the same in both male and female larvae. Those in the female larvae are slightly larger than those in the male. The mean size of the optic bud in the full-grown male larva is 232 ± 7.82 , which is 43 per cent of the size of that of the wild type (535 ± 10.14) at the same stage of development.

The mean size of the optic bud in mature female larvae is 298 ± 11.29 , or 52 per cent smaller than that of the wild type. When compared with lozenge (380 ± 5.30), Bar is 36 per cent smaller than the latter at the same stage of development. The size of larvae from which the optic buds were obtained was also compared with that of wild type and lozenge. The difference found between them proved to be statistically insignificant, indicating that they are of the same stage of development, while the difference in the size of optic buds is beyond doubt due to the effect of the particular gene concerned.

The optic bud of the wild type attains its maximum size at the end of the larval period. A little increase in size occurs in the next stage, that is, during the first four hours of the prepupal period. From one-hour Bar prepupae, the optic buds were obtained and their size determined (table 3). Their mean size in the male is 201 ± 6.11 and in the female 255 ± 10.04 , while in mature larvae it is 232 ± 7.82 for the male and 298 ± 11.29 for the female. This slight decrease in size in the prepupal period is to be accounted for as due to the compressing effect brought about by the retraction of the larva in the formation of puparium. The percentage difference in optic buds between the wild type and Bar remains surprisingly constant, Bar being 43 per cent of the size of wild type, and the same value was obtained in the mature larval stage. It was practically the same in the two stages in the female, viz., 48 per cent for the mature larva and 49 per cent for one-hour prepupae. Comparison of Bar with lozenge shows that the percentage difference is very close, Bar being 33 per cent smaller than the latter. The second value serves to check the first one for mature larvae, indicating clearly that the first value was from mature larvae. This excludes the possibility beyond all doubt that the first value might be from young larvae corresponding to the earlier stage of development in the wild type. It is evident that the effect of the Bar gene on the size of the optic bud may be definitely determined at the end of the larval period. The

failure to detect the effect of the Bar gene on the size of the optic bud in earlier stages of development suggests that the action of the Bar gene comes to its fullest expression at the end of the larval period, while in earlier stages the Bar gene may be inactive or active to a lesser degree. The detection of such an effect, if it exists, would require more accurate and refined technique. More conclusive evidence might perhaps be obtained by counting the actual number of rudiments of the ommatidia present in the optic bud. The possibilities

TABLE 3
Optic buds of Bar

BAR	SEX	NUMBER OF CASES	MEAN	S.D.	P.E. OF MEAN
Size					
Mature larva	♂	19	4.40	± .38	± .059
	♀	30	5.25	± .51	± .073
One-hour prepupa	♂	15	2.24	± .16	± .028
	♀	18	2.60	± .17	± .027
Optic buds					
Mature larva	♂	31	232	± 64	± 7.82
	♀	35	298	± 99	± 11.29
One-hour prepupa	♂	26	201	± 46	± 6.11
	♀	31	255	± 80	± 10.04

existed that either the Bar gene affects the number of rudiments of ommatidia in the optic bud and not the size of the bud, or that the Bar gene affects both the size of the optic bud and the number of rudiments of the ommatidia. The latter was found to be the case. It follows, then, that the counting technique would not help the case materially.

The evidence obtained on the action of Bar gene from a developmental point of view is in full accord with the results of physiological study. Zeleny and others have shown that the facet number in different types of Bar varies with en-

vironmental factors, such as poor food, crowding, and abnormal temperature. Krafka ('20) confirmed and extended Seyster's work ('19) in showing that temperature is effective for increasing and reducing the facet number. The effective period for temperature treatment in the life-cycle is found to be in the latter part of the larval period. From both developmental and physiological angles of attack the probability is that the effect of the Bar gene does not appear until later in the larval period and the full expression of its action occurs toward the end of the larval period.

Eyeless-4

Eyeless, an extreme eye mutant, is located in the fourth chromosome. Many 'eyeless' flies are not entirely without eyes, and there is considerable variation among these in the size of the eye. Flies of pure eyeless stock may have eyes totally lacking or they may have eyes which appear to be only a little smaller than the eyes of the normal wild type. Between these extremes all gradations may occur. Furthermore, in eyeless the symmetry between the eyes of the two sides seems to be lacking. Sometimes the eyes are about equal in size and sometimes there is a fair-sized eye on one side, while on the other there is only a small one or none at all. The variability of the eyeless condition is due, as is now known, to the influence of environmental factors, primarily to food conditions. When flies genetically pure for the eyeless gene are raised under favorable food conditions, all that emerge during the first few days are eyeless. As the stock culture gets older, more and more of the flies have larger eyes, and toward the end an increased number of the flies have both eyes present and these are almost full-sized. If some of the latter are used as the parents of a new generation, the results obtained are precisely the same as when eyeless flies are used.

The optic tracts of normal and eyeless flies were examined by Richards and Furrow ('25). They found that in the normal fly three ganglia, outer, median, and inner, connect

the eye with the brain, and in flies with small eyes all the ganglia are present, although greatly reduced. In totally eyeless flies the outer ganglion is missing and the median and inner ones are contracted into a more or less shapeless mass which, nevertheless, discloses its double nature.

Information as to the eyeless condition in the developing stages of eyeless flies was lacking. Whether the optic buds fail to form eyes because of degeneration, or whether they are wanting at the very beginning was not known.

A stock pure for the eyeless gene, called 'eyeless-4,' was used for study. This gives at first about 90 per cent entirely eyeless flies. This stock was further purified by selected in-breeding for five generations.

In very young pupae of eyeless stock (fig. 14), that is, immediately after pupation, the head is very much smaller than in the wild type (fig. 11) and is more or less compressed on both sides, apparently due to the absence of the compound eyes. The eyeless condition has been traced back to the larval period. Mature larvae were obtained from eggs laid and hatched within one hour, and raised in cultures with the most favorable food conditions. Individual larvae were first measured, the cephalic complexes, including the optic buds, were dissected out, and the size of the optic buds determined. The cephalic complex (fig. 18) showed that the optic bud is almost totally wanting, while the region anterior to the bud, including the antennal bud and the apex of the complex, seems to be approximately the same as that of the wild type, both in size and structure. The fact needs to be pointed out that, while the optic bud as a definite structure is totally lacking, there is, however, a very small portion of material present at the posterior extremity of the cephalic complex. This portion, although without an optic stalk or definite shape or structure, may be called the optic bud for the sake of convenience. The nature of this portion is not clear; probably it takes part in the development of certain dorsal portions of the head. This rudimentary condition of the optic bud is equally true in the male and in the female larvae. The sexual

difference in that respect requires further study. In table 4 it is shown that the optic bud of the male larva has the mean 107 ± 4.5 and of female, 128 ± 6.73 ; that is, in both sexes it is 20 per cent the size of that of the wild type. This value seems to be larger than would be expected for eyeless. This fact may, on the other hand, point to the possibility that a small portion of the optic bud takes part in the development of the dorsal region of the head or of the region surrounding the eyes. The antennal buds and the larval ganglia do not seem to be appreciably smaller than those in the wild type.

TABLE 4
Optic buds of eyeless-4

EYELESS	SEX	NUMBER OF CASES	MEAN	S.D.	P.E. OF MEAN
Size					
Mature larva	♂	27	4.55	$\pm .48$	$\pm .062$
	♀	25	5.17	$\pm .58$	$\pm .078$
One-hour prepupa	♂	20	2.27	$\pm .20$	$\pm .030$
	♀	20	2.64	$\pm .17$	$\pm .025$
Optic buds					
Mature larva	♂	38	107	± 42	± 4.50
	♀	42	128	± 62	± 6.73
One-hour prepupa	♂	33	58	± 24	± 2.65
	♀	31	69	± 23	± 2.90

The structure of the ganglia appears to be normal at the end of the larval period; the later stages of development have not been followed up closely enough to allow a definite opinion to be expressed. The larvae from older cultures have optic buds of varying sizes corresponding in general to the variation observed in the size of the eyes of adult flies. They are all of definite shape and structure and have an optic tract of variable size connecting the larval ganglia.

The condition of the optic buds in one-hour-old pupae was examined and found to be exactly the same as in the mature

larvae, that is, the buds are almost totally lacking. The measurement (table 4) indicates that at this stage the optic bud in the male is 58 ± 2.65 and in the female is 69 ± 2.90 —a condition even more extreme than in the larval stage. As compared with the control, the wild type of the same age, the optic bud of eyeless is only 13 per cent the size of the control in both sexes. This second value is a check on that obtained for the mature larva and is nearer to that expected.

The eyeless condition was traced back in the earlier stages of development and could be first recognized at the end of the second day, or forty-eight hours after hatching from the egg. The wild type of the same age served as the control.

TABLE 5
Optic buds of eyeless, forty-eight-hour larvae

EYELESS	SEX	NUMBER OF CASES	MEAN	S.D.	P.E. OF MEAN
Larva size					
Eyeless	♂	20	1.99	$\pm .23$	$\pm .034$
Control (wild)	♀	20	1.93	$\pm .21$	$\pm .032$
Optic buds					
Eyeless	♂	30	11	± 1.69	$\pm .207$
Control (wild)	♀	34	128	± 3.52	$\pm .407$

The cephalic complexes, including the optic buds (fig. 20), were dissected out and it was found that what has proved true for the cephalic complex in mature larvae applies equally well to the two-day-old larvae, that is, the optic bud is lacking, although a very small remnant is still present. The region anterior to the optic bud, including the antennal bud, appears to be about the same size and structure as that of the control (fig. 19). The optic bud in the wild type at this stage of development is of similar structure to that in the mature larva, only less distinctly developed, and a definite optic tract is already present. The remnant or optic bud (table 5) is only $11 \pm .207$, that is, 8 per cent the size of wild

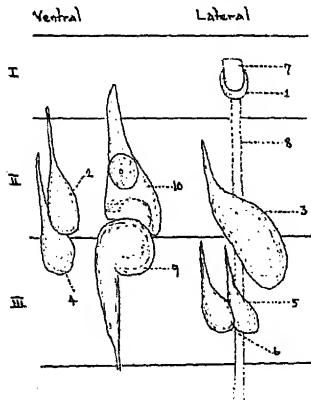
type ($128 \pm .407$). In the wild type at the end of forty-eight hours from hatching, two distinct portions of the cephalic complex have just been formed and can be recognized as such, one being the optic bud which forms the posterior region of the complex and the other, the antennal bud, anterior to the optic bud. The eyeless condition or the effect of the eyeless gene on the somatic structure concerned is to be first recognized at this larval stage of development. This is in agreement with expectation in the light of the course of development of the wild type. In the wild-type larvae of an earlier stage, forty hours old, the optic and antennal portions of the cephalic complex have not as yet distinctly separated, while in eyeless they are very much less definite. The difference in the optic region in these two types is not sufficiently great to be considered statistically, but the size of the larvae from which these two types of cephalic complexes were obtained is approximately the same, or the difference between them is insignificant. It is then clear that the effect of the eyeless gene may be identified at or before the end of the second day of the larval development.

THE THORACIC COMPLEX OF WILD TYPE

The thorax and its appendages of an adult *Drosophila* are represented at the end of the larval period by six separate pairs of distinct primordia, collectively called the thoracic complex (text fig. B). The following brief description of the position and development of these structures is given in order that a comparison may be drawn between two of them and the corresponding parts in the mutant types.

The six pairs of thoracic primordia are arranged in two groups, the ventral and the lateral, according to their position in relation to the great cephalic complex. In the ventral group there are two pairs of buds, the ventral prothoracic and ventral mesothoracic. Each of these buds is inclosed in a single sac of its own and is connected with the hypoderm of the larva by a distinct stalk, and with the larval ganglion by a nerve. The prothoracic buds beneath the cephalic com-

plex are placed very close together side by side, suggesting a single sac. The mesothoracic buds are a little posterior to the prothoracic pair and ventral to the larval ganglia. In the lateral group there are four pairs of primordia closely related to the great tracheal trunk, symmetrically arranged, with one member of the pair on each side of the cephalic complex. They are the dorsal pro-, meso-, and metathoracic buds and the ventral metathoracic buds. Each of these buds is also inclosed in a single sac, connected with the hypoderm



Text fig. B Diagrammatic sketch, showing one-half of the thoracic complex in the mature larval stage. *I*, prothorax of the larva; *II*, mesothorax of the larva; *III*, metathorax of the larva. Imaginal buds: 1, prothoracic dorsal; 2, prothoracic ventral; 3, mesothoracic dorsal; 4, mesothoracic ventral; 5, metathoracic dorsal; 6, metathoracic ventral. 7, anterior spiracle; 8, trachea trunk; 9, larval ganglion; 10, cephalic complex.

of the larva by a distinct stalk, except the dorsal prothoracic bud. In the latter case its connection with the larval ganglia is not clear, as no nerve is present. The dorsal mesothoracic pair is the largest of all the imaginal buds, more or less rectangular in shape with the anterior end, at which the stalk arises, somewhat pointed. The dorsal metathoracic pair is like the large mesothoracic pair in shape and structure, but considerably smaller in size and simpler in structure; it is located posterior and ventral to the latter. The ventral metathoracic pair is like the pair of buds in the ventral group in

structure, shape, and size, but is located lateral to the cephalic complex and a little above the midlateral line of the larva. The dorsal prothoracic buds, the most obscure of all the imaginal buds, are in the form of a thin sheath surrounding the base of the anterior spiracle on either side. Their connection with the hypoderm of the larva seems to have been broken before they were definitely attached to the spiracles.

The relation of these imaginal buds to the structure or to the organs in the thorax of the adult is given in chart 2 for the purpose of showing the relative positions of the two important primordia to be considered, in the thorax of an

CHART 2
Primordia of thorax

Primordia in mature larva		Prothoracic buds	Mesothoracic buds	Metathoracic buds
Dorsal	Definitive organs in adult	Humeri	Mesonotum Wings Scutellum	Metanotum Halteres
Ventral		First pair of legs Propleura	Second pair of legs Sternopleura	Third pair of legs Hypopleura

adult, and also their relation with other elements of the thorax. As shown in chart 2, the ventral prothoracic pair gives rise to the first pair of legs and the thoracic portion, the propleura; the mesothoracic pair, to the second pair of legs and sternopleura; and the metathoracic pair, to the third pair of legs and hypopleura. The dorsal prothoracic buds are the rudiments of the humeri, which are the small regions dorsal to the anterior thoracic spiracles at the anterolateral corners of the thorax of the adult. The dorsal mesothoracic pair gives rise to the wings and a great portion of the thorax on the dorsal and lateral sides. The dorsal metathoracic pair develops into the halteres and the small portion of thorax near the posterior end.

The dorsal mesothoracic buds in mature larvae

The dorsal mesothoracic bud in the mature larval (fig. 23) stage is somewhat rectangular, with a stalk at the anterior pointed end, connecting with the hypoderm of the larva. In the middle of the bud there are one or two transverse grooves along the whole width of the bud, thus marking off equally the anterior from the posterior portion of the bud. In the posterior portion there are four to five concentric ridges, while in the anterior the surface is more or less even. A distinct margin is present along the periphery of the whole bud, meeting in the stalk. In the center of the posterior portion there is a heavy ridge in the form of a horseshoe with the open end toward the lateral side of the bud. This ridge does not appear until the end of the larval period. It is the first sign of the wing bud, from which the wing bud is developed in the early pupal period. The anterior portion is concerned primarily with the development of the dorsolateral part of the thorax proper.

In the mature larval stage the dorsal mesothoracic bud (fig. 23) seems to have attained its definite structure and shape, and with the beginning of the wing bud has proved to be a characteristic stage with definite landmarks, with which similar buds from the wing-mutant types, such as vestigial and no-wing, may be compared. The characteristic structure in the bud at this stage of development is similar in both sexes, but the size of bud in the female is slightly larger than in the male. However, the difference in size between the sexes is significant. The mean size of the dorsal mesothoracic buds is 1310 ± 22.88 for the male and 1672 ± 39.77 for the female. The buds of the male were 78 per cent the size of those of the female. From one-hour-old prepupae the buds were dissected out and their size determined (1221 ± 14.25 for the male and 1449 ± 18.32 for the female). Again the bud of the male is smaller than that of the female. It is 84 per cent the size of the latter.

The second value shows a decrease in size compared with the measurements of the mature larva. This is to be ac-

counted for by the compressing effect brought about by the great shortening of the larva during puparium formation.

The development of the dorsal mesothoracic bud

The earliest sign of the dorsal mesothoracic bud that has been traced out was found in young larvae, sixteen hours after hatching (fig. 32). It consists of a group of small cells, uniform in size and structure, attached to the hypoderm of the larva a little below the midlateral line. Its precise mode

TABLE 6
Dorsal mesothoracic bud of wild type

WILD TYPE	SEX	NUMBER OF CASES	MEAN	S.D.	P.E. OF MEAN
Size					
Mature larva	♂	30	4.65	± .50	± .062
	♀	30	5.28	± .56	± .069
One-hour prepupa	♂	25	2.31	± .17	± .020
	♀	29	2.59	± .20	± .025
Dorsal mesothoracic					
Mature larva	♂	30	1310	± 243	± 22.88
	♀	50	1672	± 417	± 39.77
One-hour prepupa	♂	39	1221	± 132	± 14.25
	♀	41	1449	± 174	± 18.32

of origin and the time relations of its stages of development have not been accurately determined, as the interest has been rather on the mode of development after its earliest appearance. Evidence in the very nearly related forms, such as the apple maggot, *Rhagoletis* (Snodgrass, '24), and blow-fly, *Calliphora* (Lowne), shows that the dorsal mesothoracic bud, like all other thoracic buds, arises from hypodermal cells by inward growth or invagination. It may be inferred that the same is true in *Drosophila*. At the end of forty hours (fig. 30a), the bud has considerably increased in size and

attained a definite shape, i.e., the bud is almost circular in shape with a distinct, rather long stalk connecting with the hypoderm of the larva. A groove has appeared along the dorsal edge. During the next period, forty-eight hours (fig. 33a), the groove has become more distinct and extends from the stalk to the posterior end of the bud, and a notch has developed in the ventral side of the anterior portion of the bud. The bud as a whole appears to be very thin with a very even surface. During the next eight hours (forty-eight to fifty-six hours) the notch is more distinctly shown (fig. 35) and a margin of the ventral side has developed from the stalk to the posterior end of the bud. The most conspicuous change, beside an increase in size, is the appearance of a ridge at the level of and near the notch at one side extending a great portion of the width of the bud, with the two ends pointing posteriorly. This ridge marks off the bud into two regions, the triangular anterior region and the circular posterior region. It is perhaps the first sign of the concentric ridges which follow in the posterior region in later stages of development. At the end of sixty-four hours (fig. 37), one more ridge has developed posterior to the first one, and the concentric nature of these ridges is indicated, but not as yet established. The size increase in the bud seems to be proportionately the same in the two regions of the bud. At the end of the next period, seventy-two hours (fig. 39), two more ridges have developed and the concentric nature of these ridges is more or less laid down, thus leaving a central circular portion with an even surface. From this central portion the wing bud arises. At the end of the larval period, this has the form of a heavy ridge. During the last day (the fourth) of the larval period (figs. 41, 23) the change which takes place in the bud seems to be primarily an increase in size, both in area and in thickness, and the ridges become more and more distinct. The number of ridges varies from four to six and does not seem to be of much significance, as they show no direct relation to the future structures to be developed from the bud. The important event at the end of the larval period is the development of the wing bud.

Development of wing bud

At the end of the larval period, the wing bud appears as a heavy ridge somewhat like a horseshoe (fig. 23), with the open end directed toward the lateral side of the bud. It develops evidently as an outgrowth from one side of the central portion of the concentric ridges in the posterior region of the dorsal mesothoracic bud. The central portion has developed at the end of the third day, but the ridge, the first sign of a wing bud, does not appear until the end of the larval period (the latter part of the fourth day). The wing develops rapidly and is completely formed in the first five hours of the prepupal period. During the first hour the change which occurs in the bud is the growth of the ridge posteriorly and laterally. Thus, the open end of the bud has moved through 90° toward the anterior end, and not laterally (fig. 42). At the end of the second hour (fig. 43), the two lateral edges of the bud have grown anteriorly and meet at their distal ends, forming a groove between them, thus leaving behind a large portion of the bud in a two-layered pocket. The upper layer is formed from the new growth of the wing bud, while the lower layer probably comes from the original portion of the thoracic bud below the ridge. Very little change seems to have taken place in the anterior portion of the thoracic bud. By the end of the third hour (fig. 44), the region posterior to the ridge, i.e., the newly formed pocket or wing bud proper, has grown to a considerable size, and the portion in front, the original anterior portion of the thoracic bud, has also increased both in thickness and area, but has changed little in shape. At the end of the fourth hour (fig. 45), no conspicuous change in shape or other change that can be observed has occurred in the bud as a whole. During the fifth hour (fig. 46) the wing bud has grown to a considerable size and has become completely formed as a two-layered flap, being marked off from the thoracic portion, which is single-layered. At this stage of development, the fifth hour of prepupal period, the mesothoracic buds with the newly formed wing buds, along with other members of the thoracic complex, have been

brought into contact with each other in their prospective positions with reference to the regions of the thorax of the adult. This is brought about by the preliminary movement of the process of pupation, as has previously been described. By the second movement, the pupation proper, the wing bud is stretched out to form a thin flap lying flat against the side of the abdomen—the final position of wing bud in old pupae. The finer details of the integral parts of the wing structure are developed in the pupal period. The critical period of wing-bud formation, the first five hours in the prepupal period, is found to be the important stage and is used as a measure with which wing mutants are compared.

Dorsal metathoracic buds in mature larvae

The dorsal metathoracic bud (fig. 24) is essentially like the dorsal mesothoracic bud both in structure and shape, only it is smaller in size and simpler in structure. For instance, in the mesothoracic bud there are four or five concentric ridges at the posterior region of the bud, while there is only one such ridge in the corresponding region in the metathoracic bud. At the end of the larval period, it seems to have reached its definite shape and structure. The important structure developed at this stage is the halter bud. It originates, as does the wing bud, from the central portion of the concentric ridges in the posterior portion of the thoracic bud. The dorsal metathoracic bud is found, at this stage of development, to furnish definite characteristics, and therefore it is used as a standard to which the mutant type bithorax is compared. The mean size of the bud was determined to be 421 ± 8.93 for the male; and 548 ± 9.65 for the female in the mature larval stage. In a one-hour-old prepupal stage the size of the bud was 334 ± 7.35 for the male and 454 ± 7.25 for the female. The slight decrease in size of the bud in the latter stage is to be accounted for, as before, by the compressing effect of the process of puparium formation.

Development of the dorsal metathoracic bud

The mode of development of the dorsal metathoracic bud is very similar to that of the dorsal mesothoracic bud. The earliest stage of this bud was found in young larvae one day old, twenty-four hours after hatching (fig. 31), that is, eight hours later than the mesothoracic bud. The bud consists of a group of small cells (epiblast) of uniform size and structure, lying against the hypoderm below the midlateral line and in the metathorax of the larva. By the end of forty

TABLE 7
Dorsal metathoracic of wild type

WILD TYPE	SEX	NUMBER OF CASES	MEAN	S.D.	P.E. OF MEAN
Size					
Mature larva	♂	30	4.65	± .50	± .062
	♀	30	5.28	± .56	± .069
One-hour prepupa	♂	25	2.31	± .17	± .020
	♀	29	2.59	± .20	± .025
Dorsal metathoracic					
Mature larva	♂	46	421	± 89	± 8.93
	♀	45	548	± 96	± 9.65
One-hour prepupa	♂	36	334	± 65	± 7.35
	♀	40	454	± 68	± 7.25

hours, the bud (fig. 29b) has grown to a definite shape and structure resembling very closely the mesothoracic bud at the same period, except that it is much smaller. The bud is more or less circular in outline, with a slender short stalk connecting with the hypoderm at the anterior end. It was found that this is the earliest stage of development of the bud which shows definite features with which the same bud from the mutant type bithorax may be compared. Very little or no change in the bud, except increase in size, can be observed at the end of forty-eight hours (fig. 33b). By the

end of fifty-six hours (fig. 34), the margin around the bud has definitely formed, while the surface of the bud is smooth. In some cases there is an indication of the ridges soon to be formed. Indeed, in the next period, sixty-four hours, a concentric ridge has definitely formed around a central even portion in the posterior part of the bud (fig. 36) and an anterior narrow portion has also developed. During the period from fifty-six hours to the end of the larval period, the mature larval stage (the later part of fourth day), the size of the bud has increased and the concentric nature of the ridge becomes more and more distinct as development advances (figs. 38, 40). From the central portion of the concentric ridge the halter bud develops, at first as a heavy ridge. This does not begin to take place before the last stage of larval development.

Development of the halter bud

The halter bud, like the wing bud, begins to be recognized at the end of the larval period. It is completely formed during the first five hours in the prepupal period. At the end of the first hour, the bud has increased in size and become more distinct (fig. 57). It is somewhat horseshoe-shaped, with its open end toward the lateral side of the metathoracic bud. In the second hour the bud has grown anteriorly at an angle of 90° , so that the open end is now directed anteriorly (fig. 58). The growth process is rapid during the third hour (fig. 59), as the two edges of the bud have grown forward, meeting at their farther anterior ends, thus leaving a groove between them. Posteriorly, a two-layered pocket-like portion has been formed. The upper layer is the newly formed halter bud proper and the lower layer is probably the region below the ridge in the original posterior portion of the metathoracic bud. In the fourth hour the halter bud has grown to a considerable size and has assumed a definite shape (fig. 60). At the end of the fifth hour, the halter bud (fig. 61) is completely formed. The thoracic portion, which up to this time has shown little change, has now become distinctly

marked off from the posterior halter bud, and bent over toward one side. The completely formed halter bud is like a two-layered sac of two segments, the distal, small 'knob-like' segment and the large proximal one connected with the thoracic bud which is single-layered and very thin, like a sheath. At this stage, the thoracic portion with the halter bud has been brought in contact with other members of the thoracic complex in their prospective positions with reference to the thorax of the adult. This is accomplished by the preliminary movement of the process of pupation. By a second movement, the pupation proper, the thoracic portion is firmly fused with other elements of the thoracic complex and the halter bud is brought to its definite location. Further development as to the finer structures of the halter bud takes place in the late pupal period. The critical period of halter-bud formation, the first five hours in the prepupal period, was found to be the most important stage as a standard of comparison with bithorax.

THE THORACIC MUTANT CHARACTERS

In the following section the development of the various wing and thoracic mutant characters is described, viz., vestigial, no-wing, and bithorax.

Vestigial, vg

The gene for the vestigial character is in the second chromosome at 65 units.

The size of the wing is enormously reduced and this is mainly due to the trimming away of the terminal and marginal regions of the wing. Most commonly, the wing is trimmed away as far as the anterior cross vein, which in many specimens follows the new margin. The true marginal vein with its characteristic hairs is entirely removed. The basal parts of the five longitudinal veins are easily recognizable and have their normal relationship and junctures with one another. The halteres of vestigial flies are affected in a way analogous to the wings. The basal segment is little

affected, except that it is slightly shorter and smaller. The second segment is much reduced in size and in apparent complexity. The terminal segment shows the greatest reduction, becoming a barely discernible pip instead of the balloon-like segment which is the largest part of the normal balancer.

It is known that the size of vestigial wings varies within a certain range under laboratory conditions. Roberts ('18) found that high temperature increases the size of vestigial wing. He further showed that "the effect of the high temperature took place between the fertilization of the egg and the pupal stage." This result has been verified and extended in unpublished experiments carried on in the Columbia Laboratory. The critical period in which temperature affects wing length probably comes late in larval life.

The vestigial condition may be recognized in the very young pupa (fig. 22) immediately after pupation, when the vestigial wings appear as very small triangular lobes; whereas in wild type of the same stage they are large flaps somewhat rectangular in shape. At this stage of development, the shape of the wing rudiment has only just been determined; the structure is yet to be developed. The difference between the two types is very striking when they are compared side by side.

Mature larvae from pure vestigial stock, obtained from eggs laid and hatched within one hour, were reared under favorable food conditions at a constant temperature (25°C.). From them the dorsal mesothoracic buds were dissected out and their size determined in terms of square micra. A very striking difference is observed in vestigial (fig. 26). The ridge, the first sign of the wing bud, is almost entirely absent, while it is distinctly present in the wild type at the posterior end of the dorsal mesothoracic bud. The size of the mesothoracic bud is smaller in vestigial than in wild type. However, the mesothoracic bud from one-hour prepupae (fig. 47), about two hours after the mature larval stage, revealed the fact that the ridge of the wing bud has appeared and may be distinctly recognized as such. It follows, therefore, that the

appearance of the wing bud is delayed in vestigial, it being about two hours later than in the wild type. The size of the dorsal mesothoracic buds, including the wing bud, in the mature larval stage was 943 ± 13.26 for the male and 1105 ± 19.02 for the female. In the wild type it was 1310 ± 22.88 for the male and 1672 ± 39.77 for the female. Thus the vestigial male wing bud is 72 per cent, and that of the female 66 per cent the size of wild type. Yet the size of the larvae

TABLE 8
Mesothoracic buds of vestigial

VESTIGIAL	SEX	NUMBER OF CASES	MEAN	S.D.	P.E. OF MEAN
Size					
Mature larva	♂	15	4.54	$\pm .38$	$\pm .066$
	♀	18	5.17	$\pm .36$	$\pm .057$
One-hour prepupa	♂	15	2.29	$\pm .18$	$\pm .032$
	♀	21	2.65	$\pm .19$	$\pm .027$
Dorsal mesothoracic					
Mature larva	♂	26	943	± 115	± 13.26
	♀	50	1105	± 153	± 19.02
One-hour prepupa	♂	26	875	± 89	± 11.75
	♀	30	1050	± 112	± 13.79

from which these buds were obtained was approximately the same, their difference being insignificant (table 8).

From one-hour-old prepupae of the vestigial stock, the dorsal mesothoracic buds were dissected out and their size determined. At this stage, the wing bud has appeared at the posterior end of the mesothoracic bud (fig. 47). Its degree of development corresponds to that in wild type at the end of the larval period. But the wing bud of the wild type at this stage (one-hour-old prepupa) is in a much more advanced stage of development (fig. 42). This structural feature alone suffices to show the difference in the buds of these two

types. The size relations among them holds strictly in this stage of development. It was 875 ± 11.75 for males and 1050 ± 13.79 for females (table 8). In wild type it was 1221 ± 14.25 for males and 1449 ± 18.32 for females. Vestigial is again 72 per cent the size of wild type in the male and 74 per cent in the female. This second value checks perfectly with the first one obtained for the larval period. The difference in size of the pupae of these two types is insignificant (table 8). The slight decrease in size of the mesothoracic bud in the one-hour prepupal period is to be accounted for by the compressing effect in the process of puparium formation.

TABLE 9
Metathoracic buds of vestigial

VESTIGIAL	SEX	NUMBER OF CASES	MEAN	S.D.	P.E. OF MEAN
Size					
Mature larva	♂	15	4.54	$\pm .38$	$\pm .066$
One-hour prepupa	♂	15	2.29	$\pm .18$	$\pm .057$
Dorsal metathoracic					
Mature larva	♂	20	308	± 45	± 6.84
One-hour prepupa	♂	23	249	± 56	± 8.00

In the vestigial fly the halteres are affected in the same way as the wings. The dorsal metathoracic buds were obtained from mature larvae of vestigial stock. They are smaller than in the wild type, viz., 308 ± 6.84 for the male of vestigial (table 9) and 421 ± 8.93 for the wild-type male; i.e., vestigial is 73 per cent the size of wild type. The size difference in the two types of larvae from which these buds were obtained was insignificant. Structurally, there is no difference between the vestigial and wild-type buds that can be detected. In wild type the halter bud, as also the wing bud, appears at the end of the larval period. In vestigial the halter bud seems to have appeared, though it is less distinct than that in the wild type. The difficulty of determining

this point is due to the fact that a slight difference in size between the two buds is apt to be overlooked. From one-hour-old prepupae the dorsal metathoracic buds were obtained and their size determined. The halter bud seems to be of about the same stage of development as that of the wild type. The size difference holds true again in this stage, viz., 249 ± 8.00 for the males of vestigial and 334 ± 7.35 for the males of wild type. Vestigial is therefore 25 per cent smaller than wild type. The size difference in pupae of these two types is insignificant. This second value checks very closely with the first one for the larval period. Because of the lack of sufficient data it can only be inferred that the same relation exists also in the female.

An attempt was made to trace the vestigial character back in the early stages of larval development. The results obtained were not conclusive enough for certainty. The difficulty has been the lack of a definite landmark for comparison and the great variation in size and shape of buds of early stages of development.

It has been shown in normal development that the critical period of wing-bud formation is very brief, that is, the first five hours of the prepupal period (figs. 42 to 46). The rate and mode of wing-bud formation in vestigial follows step by step that of the wild type (figs. 47 to 51). The difference between them is the smaller wing bud that is formed in vestigial. The small size of the wing bud is determined by the small size of the dorsal mesothoracic bud. The process of wing-bud formation has not been altered.

No-wing

The most extreme wing character in *Drosophila melanogaster* is called apterous. The apterous fly has practically no wings at all. As this mutant character is no longer available, the next extreme wing character was used, viz., 'no-wing.' The wing reduction is only a little more extreme than in vestigial.

In the very young pupa of no-wing, immediately after pupation, the extremely small size of the wing rudiment is already apparent and may be recognized as such unmistakably. Although at this stage of development the size of the wing bud is decidedly smaller than in wild type, it does not seem to be conspicuously smaller than vestigial.

In the mature larvae from a cross between curly female and no-wing male there are two types of larvae; one develops into curly flies and the other into no-wing flies. There are no external structural features by which one type of larva may be distinguished from the other. Mature larvae of the above cross were obtained from eggs laid and hatched within one hour and raised in cultures with temperature and food conditions under control. From them the dorsal mesothoracic buds (fig. 25) were obtained and their size determined. There were two types of buds, one being distinctly smaller than the other. The small buds are beyond doubt from larvae that are going to develop into no-wing flies. The smaller buds show a structural feature which distinguishes them from those of wild type, viz., the wing bud is almost absent, while it is distinctly present in wild type. In other words, the dorsal mesothoracic buds in vestigial and in no-wing are strikingly similar in structure and shape, the difference between them being that the latter are slightly smaller than the former. The mean size of the mesothoracic buds in no-wing was 806 ± 10.26 for males and 917 ± 11.11 for females; while in wild type it was 1310 ± 22.88 for males and 1672 ± 13.77 for females, and in vestigial 943 ± 13.25 for males and 1105 ± 19.02 for females. In other words, the size of the mesothoracic buds of no-wing is 38 per cent smaller in males and 46 per cent smaller in females than in wild type, and 15 per cent smaller in males and 17 per cent smaller in females than in vestigial. The size difference in the larvae of these three types was found to be insignificant (table 10). This fact indicates clearly that these three types of larvae were probably of the same age or in the same stage of development.

From one-hour-old prepupae of no-wing, the dorsal mesothoracic buds were obtained and their size determined. At this stage, the wing bud is distinctly in a stage corresponding to that in the mature larva of wild type. It is apparent that, as in the case of vestigial, the appearance of the wing bud in no-wing is delayed, being about two hours later than in wild type. The mean size of the buds was 774 ± 14.25 in males and 914 ± 10.09 in females, while in wild type it was 1221 ± 14.25 in males and 1449 ± 18.32 in females, and in vestigial

TABLE 10
Mesothoracic buds of no-wing

NO-WING	SEX	NUMBER OF CASES	MEAN	S.D.	P.E. OF MEAN
Size					
Mature larva	♂	18	4.40	$\pm .33$	$\pm .052$
	♀	19	5.15	$\pm .39$	$\pm .060$
One-hour prepupa	♂	16	2.22	$\pm .20$	$\pm .034$
	♀	18	2.70	$\pm .25$	$\pm .039$
Dorsal mesothoracic					
Mature larva	♂	32	806	± 86	± 10.26
	♀	34	917	± 139	± 11.11
One-hour prepupa	♂	30	774	± 115	± 14.25
	♀	36	914	± 99	± 10.09

875 ± 11.75 for males and 1050 ± 13.79 for females. In other words, the size of the dorsal mesothoracic buds was 38 per cent smaller in males and 37 per cent smaller in females than in wild type, and 12 per cent smaller in males and 13 per cent smaller in females than in vestigial. The size difference in these three types of one-hour-old prepupae was again insignificant.

The attempt to trace the no-wing character back to earlier stages in larval development failed to bring to light any difference in the buds of no-wing and wild type because of the same difficulties pointed out in connection with vestigial.

Bithorax, bx

The mutant bithorax is characterized by a more or less complete change of the normal metathorax, an inconspicuous body segment behind the scutellum, into a segment like the normal mesothorax. The halteres of bithorax are nearly always conspicuously modified. They are always swollen, darkened, and hairy.

In very young pupae, immediately after pupation, from pure bithorax stock, the enlarged segment is distinctly present between the first segment of the abdomen and the scutellum on the dorsal side of the thorax. The halter rudiments seem to be also slightly larger than in wild type.

In the wild type the dorsal metathoracic bud develops into the metathorax and the halter of the adult. When the dorsal metathoracic buds of the mature larvae of bithorax were examined, they were found to be larger than the same buds of the wild type. These buds (fig. 28) were obtained from mature larvae which were raised from eggs laid and hatched within one hour under controlled conditions. The shape and structure of the metathoracic buds is essentially similar to that of wild type. Each is composed of two regions, an anterior and a posterior. In the anterior region the surface is more or less even and in the posterior region there are one or two concentric ridges, in the center of which is the distinct halter bud. The halter bud has already appeared at the end of the larval period and seems to be a little more advanced in development than that of the wild type of the same age. The mean size of the buds was 697 ± 13.94 in males and 841 ± 10.58 in females (table 11). In wild type it was 421 ± 8.93 in males and 548 ± 9.65 in females; i.e., the wild-type bud is 60 per cent in males and 63 per cent in females the size of bithorax bud.

From one-hour-old prepupae of bithorax stock the dorsal metathoracic buds were also examined and their size determined. The average was 671 ± 14.26 for males and 829 ± 13.58 for females, and in wild type it was 334 ± 7.35 for males and 454 ± 7.25 for females. In this case the wild-type bud

is 51 per cent in males and 54 per cent in females that of bithorax (table 11). The metathoracic bud of bithorax is less reduced in size during pupation than that in wild type in the one-hour prepupal stage. This fact explains, perhaps, the 9 per cent difference in the second value for the prepupal stage and also points to the possibility that it was probably a little more advanced in development than the wild type. However, the size difference between these three types of

TABLE 11
Metathoracic buds of bithorax

BITHORAX	SEX	NUMBER OF CASES	MEAN	S.D.	P.E. OF MEAN
Size					
Mature larva	♂	15	4.49	± .33	± .057
	♀	15	5.16	± .34	± .056
One-hour prepupa	♂	15	2.25	± .17	± .027
	♀	15	2.56	± .22	± .038
Dorsal metathoracic					
Mature larva	♂	28	697	± 109	± 13.94
	♀	26	841	± 80	± 10.58
One-hour prepupa	♂	24	671	± 103	± 14.26
	♀	30	829	± 110	± 13.58

pupae is insignificant (table 11), indicating that they were of the same age.

The mode of development of the halter bud in bithorax was examined. The halter bud is completely formed in rather a short time, that is, during the first five hours of the prepupal period. The mode of the halter-bud formation seems to follow that of the wild type very closely (figs. 52 to 56 and 57 to 61). Practically no difference other than the size of the bud can be detected. In the metathoracic bud there are two regions, the anterior and the posterior. The anterior region gives rise to the posterior part (metathorax) of the

thorax proper and the posterior to the halter. Now, in bithorax the dorsal metathoracic bud as a whole is considerably larger than that in wild type; thus, both the anterior and posterior regions are proportionately larger. The large size of the anterior portion makes possible the change of the normal metathorax, an inconspicuous segment, into a segment like the normal mesothorax. The large size of the halter bud gives rise to the large halter of the adult. The finer details of their structure arise in the later part of the pupal period and are not considered here, as they are not within the scope of the present study.

The bithorax character has been traced back to the early stages of larval development. This character may be first

TABLE 12
Metathoracic buds of bithorax of forty-hour larvae

BITHORAX		NUMBER OF CASES	MEAN	S.D.	P.E. OF MEAN
Dorsal mesothoracic	Bithorax	32	137	± 30	± 3.65
	Control (wild)	31	140	± 32	± 3.95
Dorsal metathoracic	Bithorax	29	107	± 23	± 2.68
	Control (wild)	31	64	± 17	± 2.84

recognized in the large size of the dorsal metathoracic bud (fig. 29b) from larvae of forty hours after hatching. Before that stage no appreciable difference in size of the buds between bithorax and wild type could be detected. At this stage of development, the shape of the bud seems to have been laid down, but no structure of any sort can be recognized. The mean size of the bud was 107 ± 2.68 (table 12), while in wild type of the same age it was 64 ± 2.84 . That is, the wild type is 56 per cent the size of bithorax. The size of the dorsal mesothoracic bud, which is located very near the dorsal metathoracic, serves as the control. Since the larvae of these two types are of the same age or the same stage of development, the mesothoracic buds should not be affected, and their size should be about the same. As expected, the

size of the mesothoracic bud was 137 ± 3.65 for bithorax and 140 ± 3.95 for wild type. It is clear that the bithorax character may be traced back to this early stage of development. Whether the dorsal metathoracic bud in bithorax appeared earlier than that in wild type is not known. This question remains to be answered.

'Giant larva'

Except for the tumor-bearing larvae, no mutant types in the larval stage had, until recently, been found in *Drosophila*. In an experiment involving III-ple female and C II III male, Bridges found a number of larvae which were larger than others in the same culture and named them 'giant larvae.' They appear late in the culture and fail to develop into adults, apparently dying in the pupal period.

The external characters of the 'giant larvae' were first examined. The larval characters of the wild type are all present and appear to be normal in location and structure. In other words, the giant larvae show no abnormality of any kind as far as their external characters are concerned. The distinctive feature of this larva is its transparency, which is greater than that of the wild type, especially in the anterior thoracic region; the internal structures in that region may be seen through the larval skin.

The internal structures of the giant larva were first examined by dissection under a binocular microscope. Histological preparations were also made for the study of microscopic structures. The mature or full-grown larvae of the 'giant' type were obtained from the F_1 of a cross between III-ple female and C II III male. From both gross and microscopic examination of these larvae it was found that all the larval organs are normal in every respect, except that they are slightly larger. All the imaginal buds are present, but they are distinctly abnormal in appearance and in structure. For example, the dorsal mesothoracic bud in the wild type (fig. 23) is somewhat rectangular in shape, with distinct anterior and posterior regions; in the former the surface is

even, while in the latter there are four or five concentric ridges and in the center of these ridges there is a horseshoe-shaped wing bud. In the giant larva the same bud is represented by a 'lump' without definite shape, whose size varies in different individuals. The anterior and posterior regions are not distinguished. The concentric ridges and the characteristic wing bud are totally absent. The same sort of abnormality is observed in other members of the thoracic complex. Again, the cephalic complex of the wild type (fig. 15) has a subtriangular sac-like appearance, with the cup-shaped optic bud at the posterior extremity and an apex at the anterior end. In the middle there is a circular antennal bud. In the giant larva the same bud appears as an elongated 'lump' without any indication of the structures which are comparable to the optic and antennal buds.

The microscopic examination of these abnormal imaginal buds revealed the fact that they show a high degree of growth. In a section through the length of the cephalic complex the whole bud appears to be perforated with holes and grooves. The cells are wholly of epiblast and are arranged in irregular branches. The nature of this type of structure is shown in figure 62. A section of the dorsal mesothoracic bud (figs. 63 and 64) shows that the irregular growth of this bud is of a different sort. Unlike the cephalic complex which is perforated, it is more or less a solid mass. Unlike the same bud in the wild type which is wholly of one type of cells, it consists of two types of cells, small and large. The small cells are apparently the original epiblast in the imaginal bud. The large cells have the structure of cells of larval tissue, especially the cells of the hypoderm of the larva. The small cells are in curved rows, instead of a homogeneous mass as in the normal case. The large cells are in rows or groups near the periphery of the bud. The nature of these large cells in the imaginal bud is not known. As this bud originates from the cells in the hypoderm, the possibility exists that they may be hypodermal cells which have been carried into the bud and have remained there undifferentiated. The con-

dition of these abnormal imaginal buds in the early stages of development has not been determined, because the young larvae of the giant type are not distinguishable from those of the normal and only a small per cent of these larvae are obtained from a cross.

In spite of the abnormality in the imaginal buds, the giant larvae pass through the larval stage in the usual way, except that the duration of this stage is slightly prolonged. When they become full-grown, they cease feeding, crawl out of the food, and come to rest on the side of the culture bottle in the same way as do the mature larvae of the wild type. Approximately two hours later, the anterior spiracles are extended, and the larval skin becomes hardened and later turns light brown in color. However, the larva, instead of becoming very much shortened as in the wild type, remains stretched, and the puparium thus formed, if it may be called such, is almost as long as the larva. Moreover, the segment with the oral hooks, instead of retreating into, projects from, the puparium. This feature, however, varies in different individuals. In this prepupal stage the degeneration of the larval tissue and of the organs is started in the same way as in the wild type. It is in this stage that the imaginal buds become developed into the rudiments of the definitive organs of the adult. In the case of 'giant' type the puparium is imperfectly formed, for the head segment fails to retreat completely and no pupa is formed. It becomes dried out afterward. The imaginal buds in this period show more extreme perforation in the cephalic complex and little or no change in the buds of the thoracic complex.

In view of these facts, it appears that in the giant larva the failure of the imaginal buds to develop into the rudiments of their definitive organs at the critical period (the prepupal period) is the cause of death. This is preceded by abnormalities in the structure of all the imaginal buds. This abnormality may be observed at or before the end of the larval period.

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In spite of the abnormality in the imaginal buds, the giant larvae pass through the larval stage in the usual way, except that the duration of this stage is slightly prolonged. When they become full-grown, they cease feeding, crawl out of the food, and come to rest on the side of the culture bottle in the same way as do the mature larvae of the wild type. Approximately two hours later, the anterior spiracles are extended, and the larval skin becomes hardened and later turns light brown in color. However, the larva, instead of becoming very much shortened as in the wild type, remains stretched, and the puparium thus formed, if it may be called such, is almost as long as the larva. Moreover, the segment with the oral hooks, instead of retreating into, projects from, the puparium. This feature, however, varies in different individuals. In this prepupal stage the degeneration of the larval tissue and of the organs is started in the same way as in the wild type. It is in this stage that the imaginal buds become developed into the rudiments of the definitive organs of the adult. In the case of 'giant' type the puparium is imperfectly formed, for the head segment fails to retreat completely and no pupa is formed. It becomes dried out afterward. The imaginal buds in this period show more extreme perforation in the cephalic complex and little or no change in the buds of the thoracic complex.

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DISCUSSION

This study of the imaginal discs of *Drosophila melanogaster* shows that most of the prospective organs of the adult can be identified in the early larval stages, but not at the same time. The order of appearance of these buds, so far as has been determined, seems to be correlated in general with the size and complexity of the organs into which they are to develop (chart 3). The cephalic complexes which give rise to a large portion of the head, including the eyes and antennae, are visible at first as a pair of 'frontal sacs' in the young larva sixteen hours after hatching. The labial buds which give rise to the small portion of the head, including the proboscis, appear in forty-hour larvae. The members of the thoracic complex show the time relation in the order of appearance more clearly. The largest pair of buds, dorsal mesothoracic, which give rise to the wings and the large portion of the thorax on the dorsolateral side, appear in larvae sixteen hours old. The three pairs of buds, which give rise to the three pairs of legs and adjacent parts of the thorax, appear in larvae thirty-two hours old. The pair of small buds which give rise to the humeri, the smallest portion of the thorax, appear latest of all, that is, not until fifty-six hours after hatching. The buds which give rise to the halteres and the metathorax of the adult appear in twenty-four-hour larvae. In the abdomen, the imaginal buds which develop into the abdominal segments are not visible in the larval stage and do not appear until eight hours after puparium formation. The buds for the external genitalia and part of the reproductive system appear earlier, that is, in young larva thirty-two hours after hatching.

The stages at which the differentiated organs appear are as follows: The optic buds, the primordia of the compound eyes, are visible in larvae at the end of the second day. The rudiments of ommatidia are laid down on the fourth day of the larval period. The various parts of the dioptric apparatus are recognizable as such on the second day of the pupal period. Elongation of these parts takes place in the

following day. The eye color begins to appear in the first part of the third day. The wing buds begin to appear as a ridge at the end of the larval period. The critical period of wing-bud formation occurs during the first five hours in the prepupal period. The integral parts of the wing structure

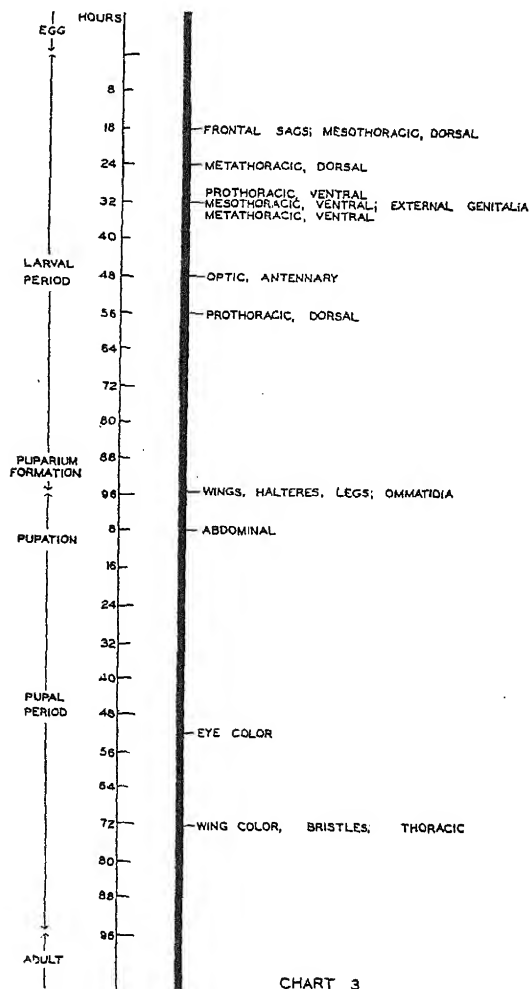


CHART 3

Chart 3

are developed in the pupal period. The color of the wings, in the folded condition, begins to appear in the four-day pupa. The halter buds follow very closely the stages of development of the wing buds. Of course, the larger the mass of cells that contributes to the formation of an organ system the earlier would it be expected to become visible macroscopically. The observations do not necessarily show that the actual time of appearance of the bud of a larger organ is earlier than that of a smaller one.

In the study of seven different mutant types of known genetic constitution of *Drosophila melanogaster*, it has been shown that their genetic differences are discernible in early stages of development. The character of each mutant type can be recognized in its early stages of development. Again, the time at which the discs of certain mutant types can be detected corresponds, to a certain extent, to the degree in which the structure is affected.

In three eye-mutant types—lozenge-3, Bar, eyeless-4—the size relation in their optic buds in the larval stage is roughly comparable to the size of their eyes in the adult stage. That is, the optic bud of lozenge-3 is smaller than that of the wild type, and that of Bar is in turn smaller than that of lozenge-3. In eyeless-4 the optic buds are practically wanting. The same relation holds true for two wing-mutant types, vestigial and no-wing. That is, the wing bud (dorsal mesothoracic) of vestigial is distinctly smaller than that of the wild type, and that of no-wing is slightly smaller than that of vestigial. This is the order of size difference in the wing in the adults of these types. In the mutant type, bithorax, the metathorax and the halter are considerably larger than in the wild type. The imaginal bud in the mature larva which gives rise to these two portions is correspondingly larger than in the wild type. In the above six cases the comparison of the imaginal buds was made in the mature larva, and the difference in the adult stages is primarily in the size of the structure concerned. In the mature larva the imaginal buds show no features except size difference, which is diagnostic. The

'giant larva,' in which there is a lethal effect on the early pupa, is unique, since in the mature larva all the imaginal buds are extremely abnormal in structure.

In the mutant types so far studied there exists a rough correspondence, at least, between the extent of the effects of a gene or genes on the structure of the adult and the stage in

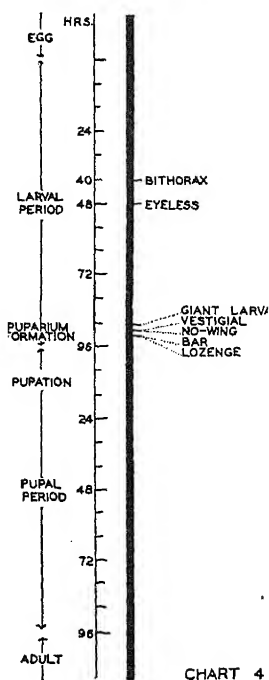


CHART 4

Chart 4

development at which the effect concerned can be recognized (chart 4). In the eye mutants, lozenge-3 and Bar, the difference has been traced back to the small size of the optic buds in the mature larval stage or possibly a little earlier. In eyeless-4, in the extreme cases, the eyes are completely lacking and the optic buds are found to be likewise lacking in the mature larval stage. Moreover, this condition has been traced to young larvae forty-eight hours after hatching. In

two wing-mutant types, vestigial and no-wing, the differences have been traced back to the small size of the wing buds in the mature larval stage or possibly earlier. In the case of bithorax the genetic difference can be recognized in the large size of the dorsal metathoracic buds in the mature larval stage, which foreshadows the bithorax condition of the adult. Again this condition has been traced back to the young larva forty hours after hatching.

In the case of 'giant larva' all the imaginal buds are extremely abnormal in structure and are pathological in nature. Here the larval period is slightly prolonged, as a result of which the larval size is likewise enlarged, but without other abnormality in structure that can be observed. The larval process seems to be normal, as is shown by the fact that the larva of the 'giant' type passes the larval period normally, but when it reaches full growth it stops feeding and crawls out of the food and becomes quiescent; the puparium is formed in the same way as in larvae in which the imaginal process is normal. The failure of the imaginal buds to assume their rôle at the critical period in pupation, due to a general abnormal effect in these buds, is perhaps the cause of death of the early pupa.

SUMMARY

1. The optic bud, the primordium of the compound eye, in the mature larva of the wild type has been examined. Its mode of development in the early larval stages has been followed, and furnishes a basis for comparison with mutant types.

2. The optic buds in the mature larval stage of three eye mutants, lozenge-3, Bar, and eyeless-4, have been compared with those of the wild type. In lozenge-3 the optic buds are proportionately smaller than in the wild type. In Bar they are distinctly smaller than in wild type and in lozenge-3. In eyeless they are almost entirely wanting, though a remaining portion is present without definite structure. The eyeless condition has been traced back to the early stages of development, at the end of the second day of the larval period.

3. The wing, in the form of a ridge at first, present as a part of the large dorsal mesothoracic bud, does not appear until the end of the larval period. The stages of development of this bud in the early larval period have been followed. The critical period of wing-bud formation was determined.

4. The wing buds of two wing mutants, vestigial and no-wing, have been examined. In vestigial the large dorsal mesothoracic bud is distinctly smaller than in wild type, and the wing bud is accordingly smaller. Moreover, the appearance of the wing bud seems to be slightly delayed. What has been found in vestigial holds true, in more extreme degree, for the mutant character 'no-wing.'

5. The halter bud, like the wing bud, arises as a ridge in the dorsal metathoracic bud. Its stages of development in the early larval period have been followed and its critical period has been determined.

6. In the mutant type bithorax the dorsal metathoracic bud was found to be larger than in wild type, and the same is true for the halter bud at the end of the larval period. This condition has been traced back to young larvae forty hours after hatching.

7. In the mature larval stage all the prospective organs of the adult are already laid down in the form of imaginal buds of definite shape and structure. In a mutant type, 'giant larva,' all the imaginal buds are present, but are extremely abnormal.

8. The general correspondence between the extent of the effects of a gene on the final structure and the stage in development at which its visible effects may be recognized is discussed.

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PLATE 1

EXPLANATION OF FIGURES

- 1 Longitudinal section through the frontal sac in wild type in sixteen-hour larva. $\times 440$.
- 2 Longitudinal section through the frontal sac in thirty-two-hour larva. $\times 440$.
- 3 Longitudinal section through incipient cephalic complex with optic stalk in forty-hour larva. $\times 440$.
- 4 Frontal sac, total, in thirty-two-hour larva. $\times 120$.
- 5 Incipient cephalic complex, total, with optic stalk in forty-hour larva. $\times 120$.
- 6 Cephalic complex, total, with distinct antennal bud (*a*) and optic bud (*b*) and the optic stalk, in forty-eight-hour larva. $\times 120$.
- 7 Cephalic complex, total, in fifty-six-hour larva. $\times 120$.
- 8 Cephalic complex, total, in sixty-four-hour larva. $\times 120$.
- 9 Cephalic complex, total, in seventy-two-hour larva. $\times 120$.
- 10 Cephalic complex, total, in eighty-hour larva. $\times 120$.

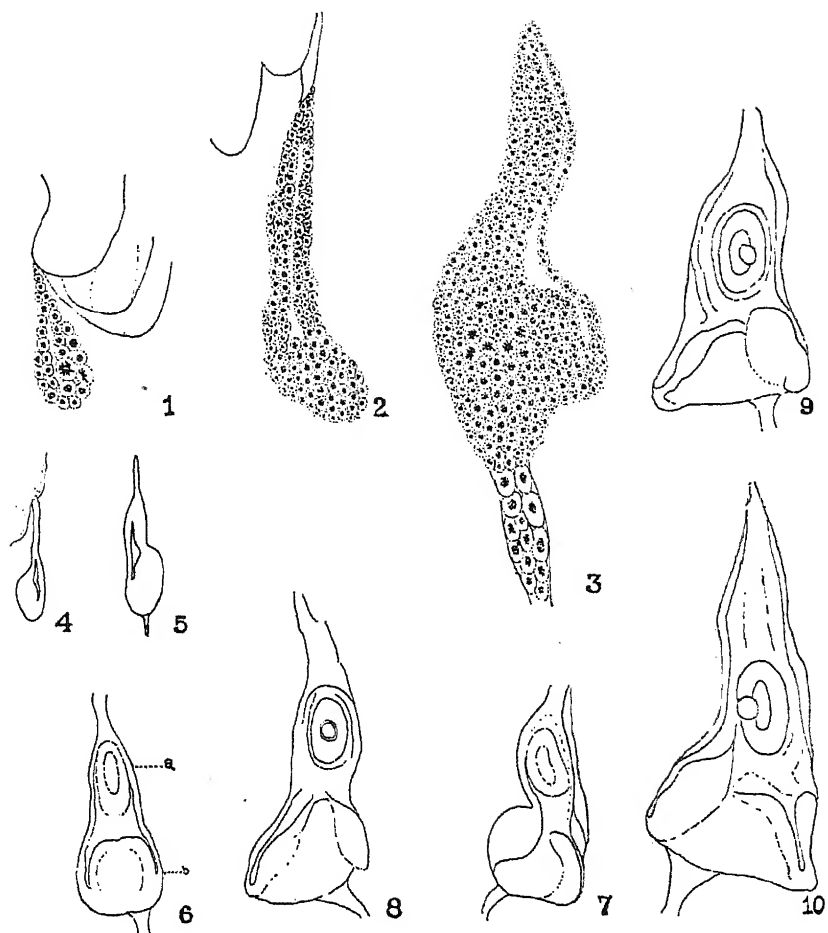


PLATE 2

EXPLANATION OF FIGURES

Figures 11 to 14 show the relative size difference in the head of wild type and three eye-mutant types.

- 11 Wild type, young male pupa, immediately after pupation.
- 12 Lozenge-3, young male pupa, immediately after pupation.
- 13 Bar, young male pupa, same age as 11.
- 14 Eyeless-4, young male pupa, same age as 11.

Figures 15 to 18 show the relative size difference in the optic buds of wild type and three eye-mutant types.

- 15 Wild type, cephalic complex, total, in mature larva. $\times 120$.
- 15a The same, in transverse section through *a* . . . *b*.
- 16 Lozenge-3, cephalic complex, total; same age as 15. $\times 120$.
- 17 Bar, cephalic complex, total; same age as 15. $\times 120$.
- 18 Eyeless, cephalic complex, total; same age as 15. $\times 120$.
- 19 Wild type, cephalic complex in forty-eight-hour larva. $\times 120$.
- 20 Eyeless-4, cephalic complex in forty-eight-hour larva. $\times 120$.

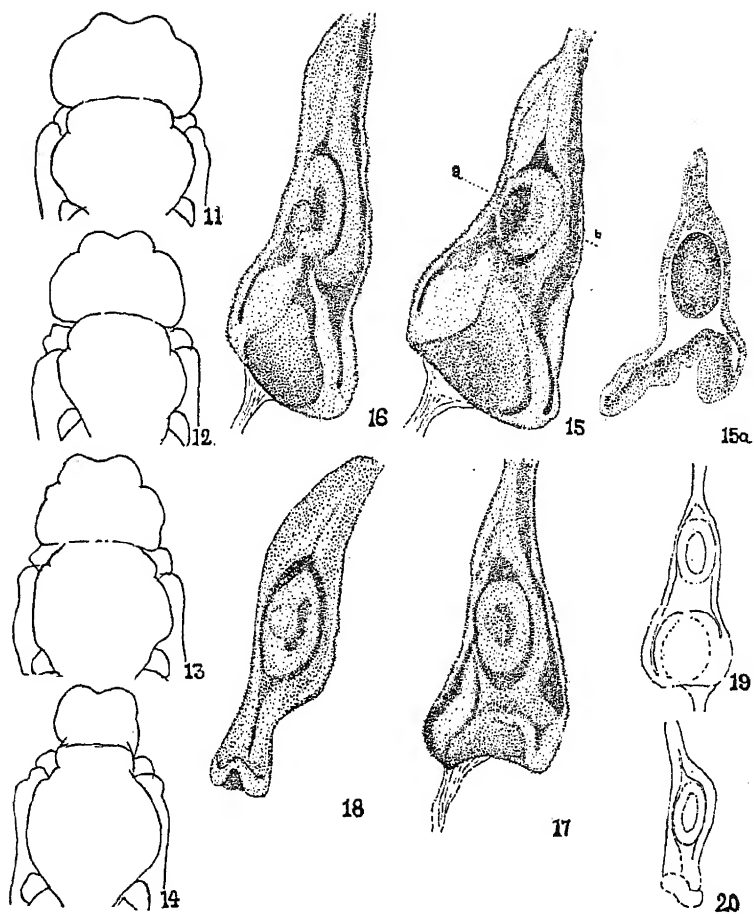


PLATE 3

EXPLANATION OF FIGURES

21 Wild type, very young pupa, immediately after pupation, showing the shape, size, and location of wing and halter rudiments.

22 Vestigial, same stage as figure 21, showing the small wing size (the halter rudiment was covered up by the wing).

23 Wild type, dorsal mesothoracic bud at the end of larval period, showing the horseshoe-shaped wing bud at the posterior region. $\times 120$.

24 Wild type, dorsal metathoracic bud, same stage as figure 23, showing the beginning of halter bud. $\times 120$.

25 'No-wing,' dorsal mesothoracic bud, same stage as figure 23, showing its smaller size and the beginning of wing bud. $\times 120$.

26 Vestigial, dorsal mesothoracic bud, same stage as figure 23, showing its small size and the beginning of wing bud. $\times 120$.

27 Bithorax, very young pupa, immediately after pupation, showing the large-sized halter and the 'lump' between the abdomen and thorax on the dorsal side.

28 Bithorax, dorsal metathoracic bud at the end of larval period (to be compared with fig. 24), showing its large size and the distinct halter bud. $\times 120$.

29 Bithorax, the dorsal mesothoracic bud (a) and the dorsal metathoracic bud (b) in young larva forty hours after hatching. $\times 120$.

30 Wild type, the dorsal mesothoracic bud (a) and the dorsal metathoracic bud (b) in young larva forty hours after hatching. $\times 120$.

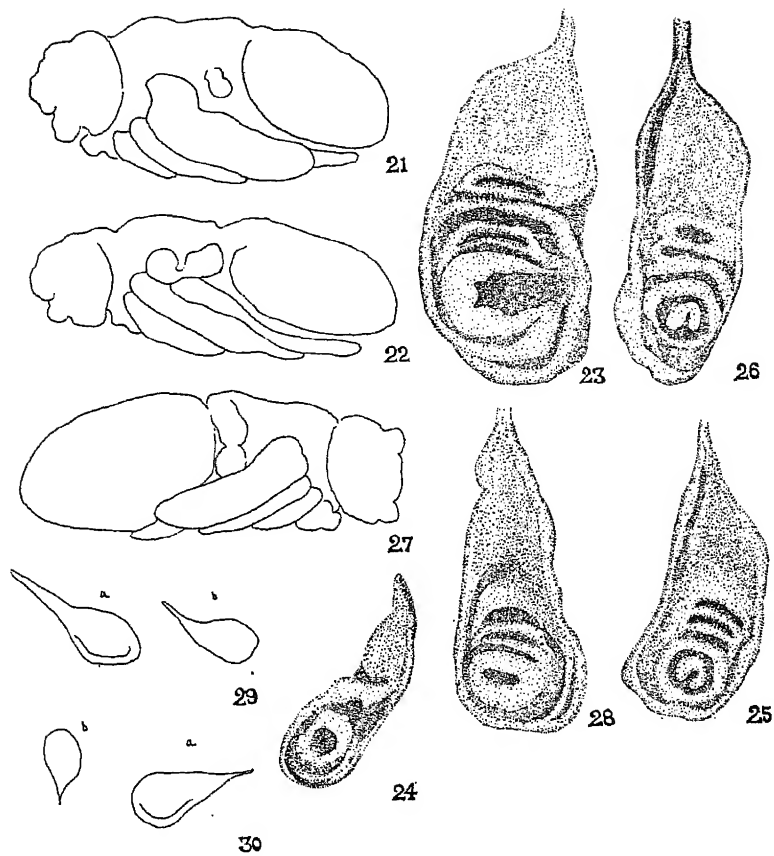


PLATE 4

EXPLANATION OF FIGURES

Figures 32, 33a, 35, 37, 39, and 41 show the developmental stages of the dorsal mesothoracic buds of wild type in the larval period.

32 The dorsal mesothoracic bud at sixteen hours. $\times 440$.

33a The dorsal mesothoracic bud at forty-eight hours. $\times 120$. (The forty-hour stage is shown in fig. 30a. $\times 120$.)

35 The dorsal mesothoracic bud at fifty-six hours. $\times 120$.

37 The dorsal mesothoracic bud at sixty-four hours. $\times 120$.

39 The dorsal mesothoracic bud at seventy-two hours. $\times 120$.

41 The dorsal mesothoracic bud at eighty hours. $\times 120$. (The last stage, eighty-eight to ninety-six hours, is shown in fig. 23. $\times 120$.)

41a The same, in section through *a*....*b*.

In the above figures note the anterior and posterior regions in the bud, the concentric ridges in the posterior region, and the wing bud in the center of the concentric ridges.

Figures 31, 33b, 34, 36, 38, and 40 show the developmental stages of the dorsal metathoracic bud of wild type in the larval period.

31 The dorsal metathoracic bud at twenty-four hours. $\times 440$.

33b The dorsal metathoracic bud at forty-eight hours. $\times 120$. (The forty-hour stage is shown in fig. 30b. $\times 120$.)

34 The dorsal metathoracic bud at fifty-six hours. $\times 120$.

36 The dorsal metathoracic bud at sixty-four hours. $\times 120$.

38 The dorsal metathoracic bud at seventy-two hours. $\times 120$.

40 The dorsal metathoracic bud at eighty hours. $\times 120$. (The last stage, eighty-eight to ninety-six hours, is shown in fig. 24. $\times 120$.)

In the above figures note the small anterior and posterior regions, the concentric ridges in the posterior region, and the beginning of the halter bud in the center of the concentric ridges.

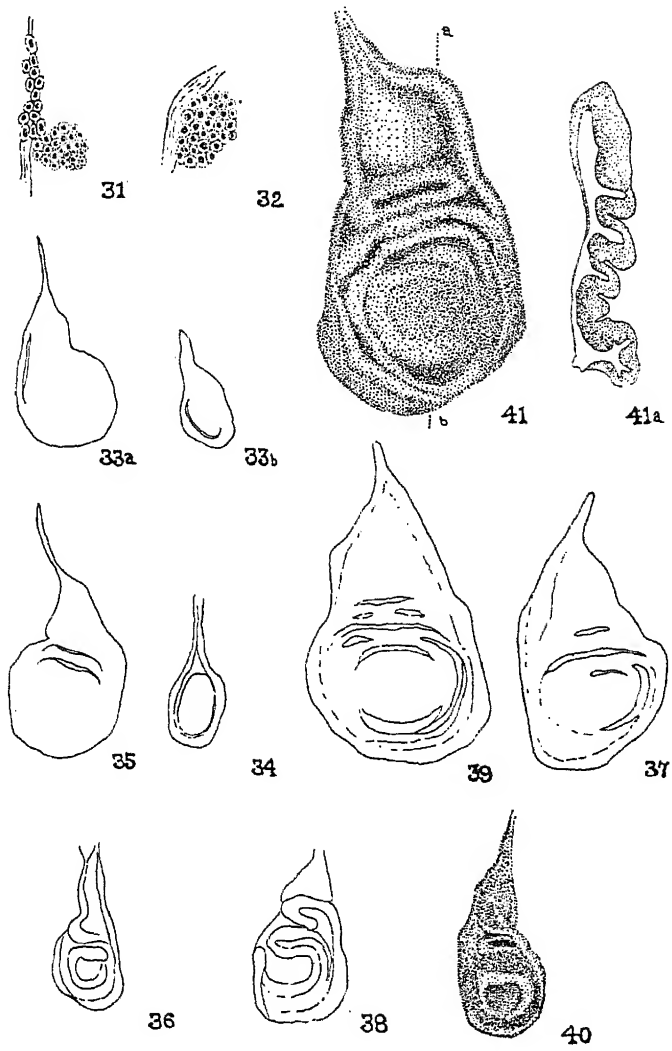


PLATE 5

EXPLANATION OF FIGURES

Figures 42 to 46 show the complete formation of the wing rudiment and the thoracic portion from the dorsal mesothoracic bud of wild type in the first five hours of the prepupal period.

- 42 Wild type, the dorsal mesothoracic bud, first hour. $\times 120$.
- 42a The same, in section through *a b*.
- 43 Wild type, the dorsal mesothoracic bud, second hour. $\times 120$.
- 44 Wild type, the dorsal mesothoracic bud, third hour. $\times 120$.
- 45 Wild type, the dorsal mesothoracic bud, fourth hour. $\times 120$.
- 46 Wild type, the dorsal mesothoracic bud, fifth hour. $\times 120$.

Figures 47 to 51 show the complete formation of the wing rudiment and the thoracic portion from the dorsal mesothoracic bud of vestigial, in the first five hours of the prepupal period.

- 47 Vestigial, the dorsal mesothoracic bud, first hour. $\times 120$.
- 47a The same, in section through *a b*.
- 48 Vestigial, the dorsal mesothoracic bud, second hour. $\times 120$.
- 49 Vestigial, the dorsal mesothoracic bud, third hour. $\times 120$.
- 50 Vestigial, the dorsal mesothoracic bud, fourth hour. $\times 120$.
- 51 Vestigial, the dorsal mesothoracic bud, fifth hour. $\times 120$.

In the figures note the small wing rudiment formed in the vestigial, and the similarity in the mode of development of the wing bud in both the wild type and the vestigial.

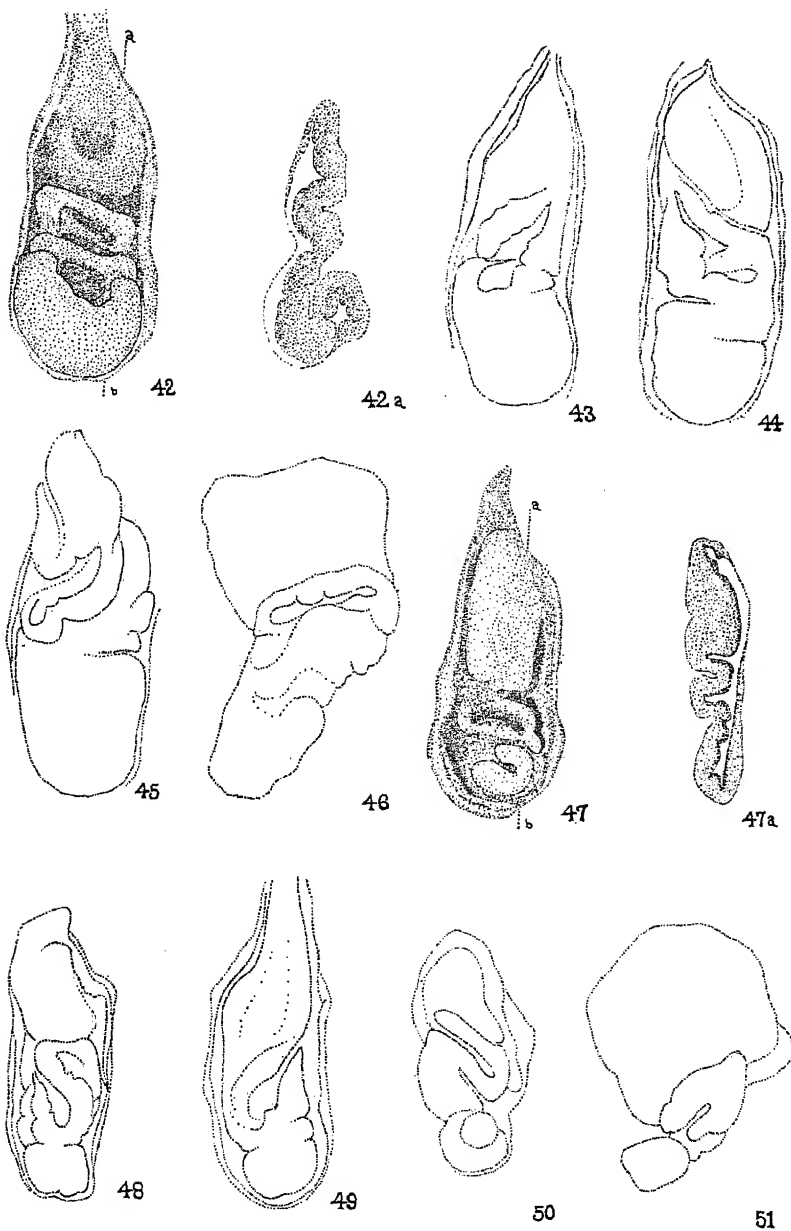


PLATE 6

EXPLANATION OF FIGURES

Figures 52 to 56 show the complete formation of the halter rudiment and the thoracic portion from the dorsal metathoracic bud of bithorax in the first five hours of the prepupal period.

- 52 Bithorax, the dorsal metathoracic bud, first hour. $\times 120$.
- 53 Bithorax, the dorsal metathoracic bud, second hour. $\times 120$.
- 54 Bithorax, the dorsal metathoracic bud, third hour. $\times 120$.
- 55 Bithorax, the dorsal metathoracic bud, fourth hour. $\times 120$.
- 56 Bithorax, the dorsal metathoracic bud, fifth hour. $\times 120$.

Figures 57 to 61 show the complete formation of the halter rudiment and the thoracic portion from the dorsal metathoracic bud of wild type in the first five hours of the prepupal period.

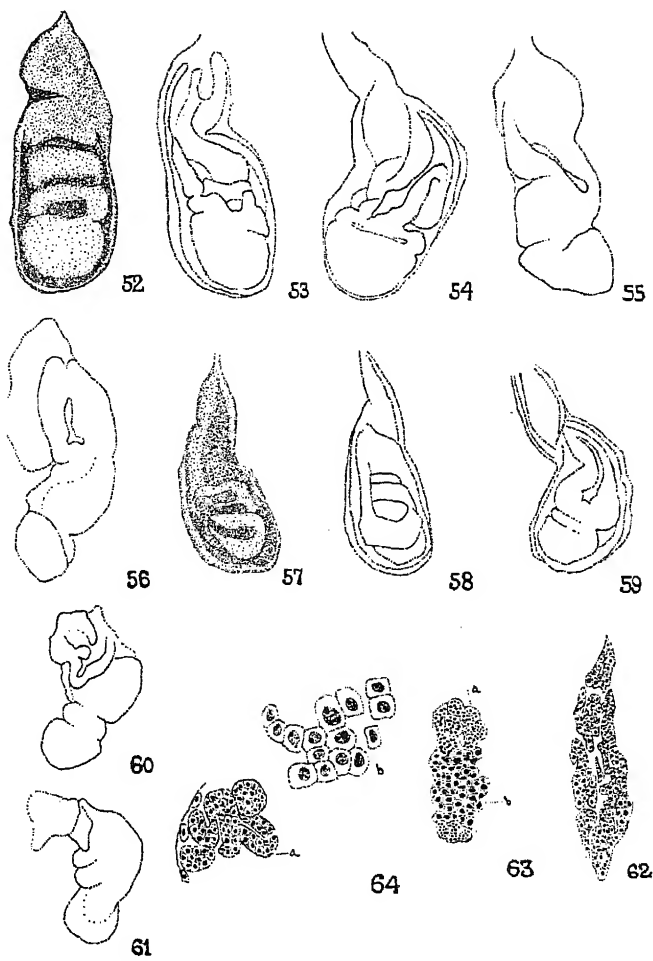
- 57 Wild type, the dorsal metathoracic bud, first hour. $\times 120$.
- 58 Wild type, the dorsal metathoracic bud, second hour. $\times 120$.
- 59 Wild type, the dorsal metathoracic bud, third hour. $\times 120$.
- 60 Wild type, the dorsal metathoracic bud, fourth hour. $\times 120$.
- 61 Wild type, the dorsal metathoracic bud, fifth hour. $\times 120$.

The above figures show the large halter rudiment and the large thoracic portion in the mutant bithorax, and the similarity in the mode of development of the halter bud in both the wild type and the bithorax.

62 Longitudinal section of the cephalic complex of the 'giant larva' in the mature larval stage, showing the extreme perforation. $\times 120$.

63 Transverse section of the dorsal mesothoracic bud in the 'giant larva' in the mature larval stage, showing two regions of the bud—*a*, the small cells; *b*, large cells—as shown more enlarged in figure 64. $\times 120$.

- 64 Enlarged view of figure 63. $\times 440$.



THE SOMATIC CHROMOSOMES OF THE OPOSSUM (DIDELPHIS VIRGINIANA)

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ONE TEXT FIGURE AND FIVE PLATES (SIXTY FIGURES)

AUTHORS' ABSTRACT

There are eleven pairs of chromosomes in the somatic cells of the opossum.
The sex chromosomes are of the x-x type in the female and the x-y type in the male.
The number and type of the chromosomes are constant in the wide variety of tissues and organs studied, except that one dividing giant cell of the spleen showed an 8n number of chromosomes.
The arrangement of the chromosomes in equatorial plates is that of an autosomal ring surrounding the centrally located sex chromosomes.

INTRODUCTION

This study was undertaken with two purposes in view: first, to ascertain the number, morphology, and behavior of the chromosomes in various differentiated tissues of a mammal and, secondly, to try out various methods of technique applicable to mammalian tissues for somatic chromosomal investigations. Examination of sections prepared in routine work for the class in histology at the Medical School of the University of North Carolina led us to consider the opossum as a favorable form. The work was begun in 1924. It was carried on at first without recourse to the literature on the subject, and this literature was not consulted until after our observations had been made. It was then seen immediately that our results accorded completely with those of Painter on the spermatogenesis and early embryology of the opossum.

The results of this work, taken in connection with that of Painter, show that the chromosomes apparently maintain an individuality throughout the life-cycle of a mammal and in all the various tissues. Studies on the spermatogenesis and

oogenesis, and on certain tissues of the embryo and of the individual shortly after birth have been made by Painter. We have been able to follow out mitoses in the later stages of development. Thus we believe that our work not only verifies his in certain details, but, also, by supplying later stages, supplements his observations.

MATERIAL AND METHODS

The material upon which this study is based consists of sections from four individuals, which were selected from a larger number of individuals of three different litters. Only one specimen (G) is from the first litter. It is a male. Three of the specimens (D, E, and F) are from the second litter and are females. The average size of the individuals of these two litters was between 50 and 60 mm. (crown-rump measurement). The length of the individuals of the third litter was about 20 mm. No figures from material of the third litter are included in the plates.

The tissue was fixed in Bouin (saturated aqueous solution of picric acid, 75; formalin, 20; glacial acetic acid, 5 parts), strong Flemming-urea cold, Carnoy-Lebrun, and Allen's modification of Bouin. The organs were dissected from the bodies and placed in the fluids, in the case of the second litter. The individuals of the first and third litters were placed directly in the fixing fluids after the skull and body cavities had been opened. Plain Bouin and Allen-Bouin were the most satisfactory fixatives.

One interesting observation was made in connection with the methods of fixation. Opossum G remained in Bouin for several months and was undamaged, apparently, by its long stay in the fixative. This suggests a possible method applicable to field or exploration work.

The material was run up in gradual changes of alcohol, although neither the drop method nor the 1-per-cent-at-a-time change was employed. It was finally dehydrated and cleared by a number of methods: 1) From 70 per cent alcohol to anilin to methyl salicylate to paraffin. 2) From 95 per cent

to cedar oil to xylol to paraffin. 3) From absolute to absolute-chloroform to chloroform to paraffin. 4) From absolute to absolute-xylol to xylol to paraffin. The first and third of these methods are probably the preferable ones.

Sections were cut mostly at 7, 8, and 10 μ in thickness, the thinner sections being, on the whole, preferable. They were stained in iron-haematoxylin (Heidenhain's), Mallory's phosphotungstic acid-haematoxylin, and Delafield's haematoxylin and eosin. The first two methods are the best. The second is particularly advised as a stain to accompany iron-haematoxylin-stained sections, for the progressive destaining of the latter is avoided and it is usually much easier to count superimposed chromosomes than with the iron-haematoxylin method. While it colors the chromosomes deeply, it leaves them more transparent than does iron-haematoxylin.

We have found it advisable to make up fresh iron-haematoxylin for each batch of slides, particularly if the previously used stain had become muddy. Only the commission-certified stain should be used, and the solution of iron alum should be fresh. The percentages of the latter solutions are given by Painter ('24).

Sections were mounted in balsam, dammar, and diaphane. The latter is particularly useful and convenient to work with.

While the methods outlined above will no doubt be modified from time to time, they have yielded us in many cases what we consider brilliant equatorial plates. There is no substitute, however, for the laborious process of searching tissues for suitable mitotic figures.

The young opossum is cordially recommended as a mammalian form easy to obtain, with a small number of chromosomes which are capable of being demonstrated clearly and with a reasonable amount of ease.

THE NUMBER OF CHROMOSOMES

With the exception of a giant cell of the spleen which will be discussed further on, the number of chromosomes in the somatic cells of the opossum is twenty-two. This verifies the

count of twenty-two in spermatogonia and dividing cells of the embryonic central nervous system of both male and female embryos made by Painter ('22). A number of years ago, Jordan ('11) reported seventeen as the number in the spermatogonia of the opossum, and Hartman ('19) reported twelve as the haploid number of the second maturation spindles of ova. The error in Jordan's count was likely due to the cruder technique in vogue during that earlier period of chromosome study, and the error of Hartman was due, as shown by Painter, to the precocious division of one of the tetrads.

Our counts have been made in both male and female young, and in both sexes, wherever a count has been possible, we have found (with the exception noted above) twenty-two chromosomes. Dividing cells have been studied from a large number of different organs, including the brain, tongue, lung, thymus, liver, intestine, pancreas, spleen, kidney, adrenal, and the connective-tissue coats of arteries. Altogether, more than 200 mitotic figures have been studied, and so we feel no hesitancy in asserting that the somatic number is twenty-two. This is also the diploid number of the germ cells as was determined by Painter.

One of the most important methods employed in studying the number of chromosomes is to draw the chromosomes of a given complex in linear arrangement (figs. 1 to 36). This method was employed by one of us (Hoy, '16, '18) in the study of somatic chromosomes of certain insects, and has been used by others as well. Its importance has been emphasized by Painter in a number of his papers. As will be noted further in the next section, there is a uniformity of types of the chromosomes, and a knowledge of the types is a wonderful aid in enumerating the chromosomes of any complex.

THE TYPES OF CHROMOSOMES

The determination of the types of chromosomes is a very important phase of somatic-chromosome study, and it is surprising how readily one comes to recognize the various chromosomes which occur in an equatorial plate.

In the first place, there are two general type groups of chromosomes in the opossum, one characteristic of the female and one of the male. The former is characterized by the presence of two x-chromosomes, the latter by an x-y pair (see figs. 1 to 24 for the female type, figs. 25 to 36 for the male type). The x-chromosomes are the smallest of all the chromosomes, with the exception of the y-chromosome, which is considerably smaller than its mate, the x. Because of their position, as will be explained further on, there is no difficulty in picking out of an equatorial plate these x- and y-chromosomes. The determination of the sex from the slides was verified subsequently in every case by examination of the external genitalia, the bodies of the opossums having been preserved.

In the second place, every chromosome group shows that the chromosomes occur in pairs with regard to length. In some of the figures a slight discrepancy in length will be noted between members of a pair, such as pair *a* in figure 5, pair *b* in figure 11, or pair *c* in figure 36. These discrepancies can be explained on the basis of the angle assumed by the chromosomes in the particular group or, as is very likely in some cases, the error involved in camera drawings at high magnifications. The members of pair *c* in figure 4 show a marked discrepancy in length as represented. This is due to the fact that the left chromosome of this pair occupied an almost vertical position in its group, as could be demonstrated by altering the focus. It is important to stress the fact that the chromosomes occur in pairs with respect to length. The reasons for the somewhat varying shape and also the width will be explained further on. There seems now no need to defend the theory that the chromosome complexes are made up of pairs. This subject is adequately treated in Parmenter's ('19) paper based on a detailed and careful metrical analysis of the chromosomes of *Ambystoma*.

In the third place, three pairs of chromosomes in each complex are noticeably larger than the others (pairs *a*, *b*, *c*, figs. 1 to 36), and seven pairs of chromosomes of gradually dimin-

ishing size lie between the group of large chromosomes and the sex chromosomes (pairs *d, e, f, g, h, i, j*, figs. 1 to 36).

The large chromosomes may be called macrochromosomes (M) and those intermediate in size, the mesochromosomes (meso)—following the nomenclature employed by Hoy ('16, '18) in his studies of the somatic chromosomes of certain insects. Thus the formulae for the somatic chromosomes in the opossum are as follows:

$$\begin{aligned} 6\text{ M} + 14\text{ meso} + 2x &= \text{female} \\ 6\text{ M} + 14\text{ meso} + x + y &= \text{male} \end{aligned}$$

Note that this formula is carried out not in one organ or in particular cells of some one group, but that it is expressed in dividing cells of at least eleven well-differentiated tissues and organs. Moreover, it is not the expression of the dividing cells of one individual only, but is characteristic of all four of the individuals studied, the single male and the three females agreeing in all respects, except that the male, instead of having two x-chromosomes, has a much smaller y- mated with a single x-chromosome.

THE SHAPE OF THE CHROMOSOMES

We have established the facts concerning the number and the types of pairs of the somatic chromosomes. Are there any peculiar shapes by which certain of the chromosomes can be identified, as can be done in the domestic fowl (Hance, '26) and in the Indian runner duck (Werner, '27)? This question can be answered in the negative. All the chromosomes of the opossum are more or less rod-like, showing evidence, especially in early prophases, of considerable flexibility. As a rule, when the chromosomes are assembled in the equatorial plates, they are not bent, or only slightly so, with the exception of those in very small cells such as the thymus (fig. 34). The chromosomes can be distinguished from one another only by length.

Reference to figures 1 to 36 will show that certain members of many pairs are apparently much broader than their mates. This is due to the fact that the longitudinal division of the

chromosomes is beginning to take place—a phenomenon incapable of correct representation in line drawings. As a matter of fact, these chromosomes frequently show a light area extending down the length through the middle which marks the thinning out of the chromatin as the halves are separating. This division begins in the distal end (i.e., the end of the chromosomes away from the central point of the equatorial plate) and results in the bilobed appearance of many of the chromosomes at that end. Many of the chromosomes show an attenuated proximal end, and, frequently, these attenuated ends show a rounded knob-like structure. Because of the fact that many of the chromosomes of a given complex may show preliminary (or precocious) splitting, it is obviously impossible to measure the chromosomes except by the single standard of length.

One peculiarity is sometimes shown by the x-chromosomes. Instead of being short, plump rods, they may show a bilobed appearance (figs. 4, 7, 16, 20). This does not mean that division in the metaphase is transverse instead of longitudinal in these chromosomes, for all the evidence is to the contrary. The right-hand x-chromosomes in figures 2 and 17 which show precocious splitting exhibit the characteristic longitudinal splitting, and this is the evidence furnished in every case where the chromosomes are dividing. This bilobed appearance of the x-chromosomes is commented on by Painter ('25).

THE BEHAVIOR OF THE CHROMOSOMES IN EQUATORIAL PLATES

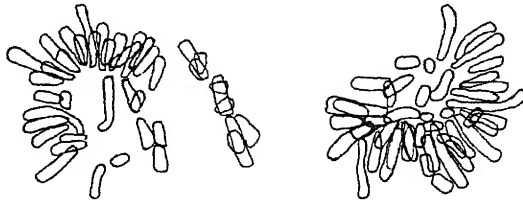
One of the striking features of equatorial plates is the almost invariable position of the x- and y-chromosomes. Figures 37 to 48 show equatorial plates from the dividing cells of three females, and figures 49 to 60 show equatorial plates from a male. In every case the sex chromosomes lie in the central portions of the plate, though this position is somewhat one-sided in figures 47 and 54. This position of the x- and y-chromosomes makes it possible, after the types of pairs have been studied, to recognize at a glance the sex of the individual from which the tissue was obtained.

Another, though not an invariable, feature is the proximity of members of a pair (judged by the criterion of length). Also, one of the smaller mesochromosomes is frequently associated with the sex chromosomes in the central portion of the plate (figs. 44, 46, 48, 49, 50, 51, 52, 57). Since the relative size of these central mesochromosomes varies in different plates, this is probably a chance association. The presence of two of the larger mesochromosomes in the center of the plate in figure 59 is most probably accidental, or rather an artifact due to technique. The pull of the microtome knife is sufficient to account for this. We have noticed in many cases where the plates have been divided by the knife that there is considerable scattering of the chromosome in one or the other of the adjacent sections.

The question of the gonomeric grouping of the chromosomes remains to be considered. It has been suggested that there is a tendency in equatorial plates for the paternal chromosomes to become grouped on one side of the plate and for the maternal chromosomes to become grouped on the other (lately discussed by Werner, '27). We doubt the truth of this hypothesis when actually applied to all equatorial plates. There are some which might be interpreted as showing this, as, for example, figures 37, 39, 40, 41, 46, 55. On the other hand, there are others where the 'division line' separating the two groups must be considerably distorted in order to separate them. The conception of gonomery is completely at variance with the fact of the association of pairs seen in some insects and frequently seen in the opossum. Metz ('16), in eighty specimens of *Diptera*, found this pairing not only in the sex cells, but also in the somatic cells. This pairing occurred, moreover, in all stages of cell division and persisted throughout the life-cycle of the individual from the egg to the adult.

A MULTIPLE-CHROMOSOME GROUP IN A GIANT CELL
OF THE SPLEEN

Text figure A shows the chromosomes of an equatorial plate in a giant cell of the spleen of a male opossum. This was drawn from two sections. The knife cut the plate in two, and in cutting apparently dragged a small group of chromosomes toward the right of the left section. Note the arrangement of the macrochromosomes and of the mesochromosomes around the periphery of the figure, as is the case in an ordinary equatorial plate showing the diploid figure. Note also the presence of at least three y-chromosomes in the central portion of the plate in the section to the right; also, the presence of what are probably x-chromosomes in the central



. Text fig. A Equatorial plate in a giant cell of spleen from male opossum. Drawn from two sections.

portion of each section. While it is not possible to count with complete accuracy the chromosomes of this figure, we can distinguish about eighty-six. If this is true, then we are dealing here with a polyploid number of chromosomes or, in other words, an $8n$ complex. It is the only figure we have found, and, therefore, not much can be said concerning mitoses in these giant cells. There are at least eight chromosomes noticeably larger than the others. This bears out the probability of an $8n$ number, since the diploid number has but one pair of these largest macrochromosomes.

The origin and significance of multiple-chromosome groups remains obscure. They occur in other tissues of a number of species, such as giant spermatogonia in the lizard, opossum, and man (Painter, '21, '22, and '23), and the pig (Hance,

'17 a). They have also been reported from a number of tissues in insects (for discussion see Hoy, '16, and Holt, '17). The origin of such groups might be the fusion of cells, the suppression of cytoplasmic division in mitosis, or, in the case of polyploidy, repeated splitting of the chromosomes. At any rate, the view expressed (Hoy, '16, pp. 338, 339) still holds good, namely, that "whether these cells are degenerating or are highly specialized is of course problematical, but they do not affect the view that the normal somatic chromosome number is a constant one. . . ."

A comparative study of these multiple-chromosome groups, the origin and fate of these cells in question would be an important contribution to our knowledge.

THE CHROMOSOMES OF MARSUPIALS

The chromosomes have been studied in ten species of marsupials. These include three species of the Polyprotodontia: *Didelphis virginiana* with 22, *Dasyurus maculatus* with 14, and *Sarcophilus ursinus* also with 14; and seven species of the Diprotodontia: *Macropus ualabatus* and *Potorous tridactylus* with 12 each, *Phascolomys mitchelli* with 14, *Phascolarctos cinereus* with 16, *Pseudochirus peregrinus* and *Trichosurus vulpecula* each with 20, and *Petauroides volans* with 22. (See papers by Agar, '23; Greenwood, '23, and Altmann and Ellery, '25.)

All of the above agree in having the x-y chromosomes in the male. Agar ('23) believes that in *Macropus* the x-chromosome is attached to some other chromosome, since it is rarely seen as a separate entity in spermatogonial mitoses, and that the x-y bivalent is more often attached to some autosome in the meiotic division.

It is impracticable to draw many conclusions from the reports on marsupial chromosomes, for in the above cases, with the exception of the opossum, only the number of the chromosomes and the type of the 'sex' chromosomes interested the investigators. A more detailed study of the types and arrangement needs to be made, and it is hoped that this

will be done for the Australian marsupials. However, from the published figures it can be seen that the x- and y-chromosomes in the males, and the x-chromosomes in the few figures of female cells occupy the center of the equatorial plates, as they do in the opossum. This seems to be characteristic of the group. There are also several pairs of macrochromosomes figured in the plates of the various species. Certain of the species (*Macropus*, *Phascogale*, *Sarcophilus*, and *Dasyurus*) show the presence of large and small U- and J-shaped chromosomes, which would indicate atelomitic attachment of the spindle fibers, whereas the presence in the remaining species of long or short rod-like chromosomes indicates telomitic attachment. A somewhat similar condition is reported by Pincus ('27) where large U-shaped chromosomes indicating atelomitic spindle attachment were found in the black rat, whereas the Norwegian rat lacked these. In the latter the spindle-fiber attachment was telomitic.

One interesting feature is the bilobed condition of the x-chromosomes seen especially in the figures of the equatorial plates in *Sarcophilus* and *Dasyurus*. Painter ('25) notes this. We have found this as a frequent, though not invariable, feature of the opossum x-chromosome.

In all cases the x- and y-chromosomes are the smallest and, as is to be expected from similar comparative studies in the insects, the y-chromosome ranges in size from that of nearly equal to the x in *Potorous* to a small 'chromatic dot' in the forms reported by Greenwood. Here again a similar condition exists in two species of rats, as reported by Pincus. In the black rat the y-chromosome is the smallest of the entire complex, but in the Norwegian rat it is as large as some of the mesochromosomes.

Painter ('25) has suggested that the total amount of chromatin and not the number of chromosomes is of primary significance and, in reviewing Agar's ('23) paper, points out that, in *Macropus*, where the number of chromosomes is twelve, there is in amount a "rough equivalent of the opossum autosomal complex" (p. 390).

DISCUSSION

From the standpoint of genetics, the careful and accurate study of the chromosomes is of especial importance, since it offers the accepted cytological basis for such studies. In a recent address Little ('28) calls attention to the need for studying the genetics of various mammals. Acknowledging the debt which the science owes to the painstaking researches of many investigators in *Drosophila*, and without disparaging their work in the least, he maintains that "there is a wide and somewhat awesome gap between the details of the structure of the germ cell in *Drosophila* and the wise direction of a program of improvement in human biology" (p. 22). In connection with experimental work and the collection of data on mammalian genetics, he calls attention to the need for studying mammalian cytology, suggesting that "it would not be at all surprising if in mammals the internal organization of the germ cell itself including the interrelationship between cytoplasm and chromosomes and between chromosomes themselves were much less definite and predictable than those of insects" (p. 23). In substantiation he calls attention to the report of Miss Swezy ('27), who reports two races of rats, one with forty-two and one with sixty-two chromosomes.

We believe that for one mammalian species, namely, the opossum, we have answered the above questions on the chromosomes. So far as we can see, the behavior of the chromosomes in the cells of various tissues of the opossum differs in no radical fashion from that of insects such as *Anasa*, *Diabrotica*, and *Epilachna*. There are in all of these forms certain definite size relations maintained among the chromosomes, these chromosomes occur in pairs, and the cells of various tissues show the same general complex. In the opossum the chromosomes are more or less regularly arranged in the equatorial plate in an autosomal ring of twenty chromosomes with the x and the y in the center. Apparently, no such striking regularity in arrangement is evident in the insects, except for the *Diptera*.

The only other mammal that has been studied from the standpoint of somatic chromosomes is the pig, though the dividing cells of the amnion are being studied in a number of forms (see Painter's papers). In the pig, Hance ('17) reports fragmentation of the chromosomes in the somatic cells. Painter ('26) suggests that this may possibly be due to delayed fixation.

Aside from its importance as a foundation for genetics, the study of the chromosomes is of great interest from the standpoint of taxonomy and evolution. McClung ('17) states that just as the taxonomist reaches certain conclusions regarding relationship by careful study of external characteristics, distribution, etc., so the cytologist can independently arrive at similar conclusions based on a study of the chromosomes. The relation between chromosome number and type on the one hand and taxonomy and evolution on the other has been worked out by McClung ('09) for the Orthoptera, by Painter ('21) for the lizards, and by Metz ('14, '16 a, '16 b) for the Diptera. An interesting and striking comparison is that of the microchromosomes of the lizards and the microchromosomes of the domestic fowl (Hance, '26) and the duck (Werner, '27).

Painter ('25), after a study of representatives of most of the eutherian orders, concludes that forty-eight chromosomes is the typical number for the Eutheria, and that when a number in some species differs from this the condition is due either to fragmentation or end-to-end fusion. He also suggests that the total amount of chromatin represented in a chromosomal complex of the opossum is the equivalent in amount of the chromatin in a similar eutherian complex.

SUMMARY AND CONCLUSIONS

1. There are eleven pairs of chromosomes in the somatic cells of the opossum.
2. The chromosomal complex of the male opossum is made up of six macrochromosomes, fourteen mesochromosomes, one x- and one y-chromosome which are the smallest.

3. The chromosomal complex of the female opossum is made up of six macrochromosomes, fourteen mesochromosomes, and two x-chromosomes which are the smallest.

4. One pair of macrochromosomes in both the male and the female complex is larger than the other two pairs. The mesochromosome pairs show a gradual decrease in size down to the seventh pair.

5. The number and type of the chromosomes are constant in all the somatic cells studied, which include glomerular, connective-tissue, and epithelial cells of the kidney, cells in the tunica fibrosa of the kidney, epithelial cells of the adrenal, cells in the cerebrum, connective-tissue cells of the intestine, epithelial cells of intestinal glands, epithelial cells of the lung, hepatic cells, pancreatic cells, connective-tissue cells of the pancreas, splenic cells, cells of the thymus, and cells from the tunica externa of arteries.

6. In equatorial plates of all somatic cells, the invariable arrangement of the chromosomes is that of an autosomal ring surrounding the two x-chromosomes or the x- and y-chromosomes. Thus the sex of the individual from which the preparation was made can be determined at a glance.

7. One dividing giant cell of the spleen showed an $8n$ number of chromosomes.

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EXPLANATION OF PLATES

All figures were drawn at a magnification of approximately 3300 diameters and are reproduced here at a reduction of one-third in size. The drawings were made with a camera lucida, and the optical equipment consisted of a Leitz 1/12a semi-apochromatic oil-immersion lens and a B. & L. no. 20 hyperplane ocular.

PLATE 1

EXPLANATION OF FIGURES

All the figures on this plate are from opossum D, a female, and represent a linear arrangement of the chromosomes. Figures 2 and 4 are from material fixed in Bouin, and dehydrated and cleared through absolute and xylol. All the other figures are from material fixed in Allen-Bouin and dehydrated and cleared through anilin-methyl salicylate. All sections were stained with iron-haematoxylin.

1 From an equatorial plate in a cell of a glomerulus of the kidney.

2 to 4 From equatorial plates in connective-tissue cells, cortex of the kidney. Figure 2 drawn from figure 39; figure 3 from figure 37.

5 to 8 From equatorial plates in connective-tissue cells of the intestine. Figure 6 drawn from figure 40; figure 7 from figure 41. The chromosomes of figure 8 lie in two sections.

9 and 10 From pre-equatorial stages in epithelial cells of the adrenal. Each drawn from two sections.

11 and 12 From equatorial plates in cells of the cerebrum. Figure 11 drawn from two sections.



PLATE 2

EXPLANATION OF FIGURES

Figures 13 to 18 are from opossum E, a female, and represent a linear arrangement of the chromosomes from tissue fixed in Allen-Bouin (with the exception of fig. 15), dehydrated and cleared through anilin-methyl salicylate, and stained in iron-haematoxylin. Figure 15 is from material fixed in Carnoy-Lebrun, with the same subsequent treatment as the foregoing tissues, but stained in Delafield's haematoxylin and eosin.

Figures 19 to 24 show the linear arrangement of the chromosomes from dividing cells of opossum F, a female. Figures 19 to 21 and 23 are from tissue fixed in cold Flemming-urea and cleared through cedar oil and xylol. Figures 22 and 24 are from tissue fixed in Carnoy-Lebrun and run up through absolute and xylol. All figures from opossum F were stained with iron-haematoxylin.

13 From an equatorial plate in an epithelial cell of a renal tubule. Drawn from figure 43.

14 From an equatorial plate in a connective-tissue cell, cortex of the kidney. Drawn from figure 44.

15 From an equatorial plate in a cell of the lung. Drawn from figure 45.

16 to 18 Connective-tissue cells of the intestine. Figures 16 and 18 are from pre-equatorial prophase and each is drawn from two sections. Figure 17 is from an equatorial plate.

19 From an equatorial plate in an epithelial cell of a renal tubule. Drawn from figure 46.

20 From an equatorial plate in a connective-tissue cell, cortex of the kidney.

21 From a pre-equatorial prophase in a connective-tissue cell of the intestine.

22 From an equatorial plate of an epithelial cell of an intestinal gland. Drawn from figure 47.

23 and 24 From connective-tissue cells of the intestine. Figure 23 is from a pre-equatorial prophase and is drawn from two sections; figure 24 is from an equatorial plate and is drawn from figure 48.



PLATE 3

EXPLANATION OF FIGURES

All figures on this plate are from opossum G, a male, and represent a linear arrangement of the chromosomes. All tissues were fixed in Bouin's. Figures 26 and 27 are from tissue given an after-treatment of Zenker's acetosublimite. Figures 25, 35, and 36 are from sections stained with phosphotungstic acid-haematoxylin. All others are from sections stained with iron-haematoxylin.

25 From an equatorial plate in an epithelial cell of a renal tubule. Drawn from two sections.

26 From an equatorial plate in a connective-tissue cell, cortex of the kidney. Drawn from figure 49.

27 From a pre-equatorial prophase in a cell of the tunica fibrosa of the kidney. Drawn from two sections.

28 From an equatorial plate in a hepatic cell. Drawn from figure 55.

29 From an equatorial plate in a pancreatic cell. Drawn from figure 56.

30 From a pre-equatorial prophase of a connective-tissue cell of the pancreas. Drawn from two sections.

31 From a pre-equatorial prophase of a cell in the spleen.

32 From a pre-equatorial prophase in an epithelial cell of the adrenal. Drawn from two sections.

33 and 34 From equatorial plates in dividing cells of the thymus. Figure 34 drawn from figure 58.

35 From an equatorial plate in a cell from the tunica externa of a branch of the pancreatic artery.

36 From a pre-equatorial prophase in a connective-tissue cell of the pancreas. Drawn from two sections.

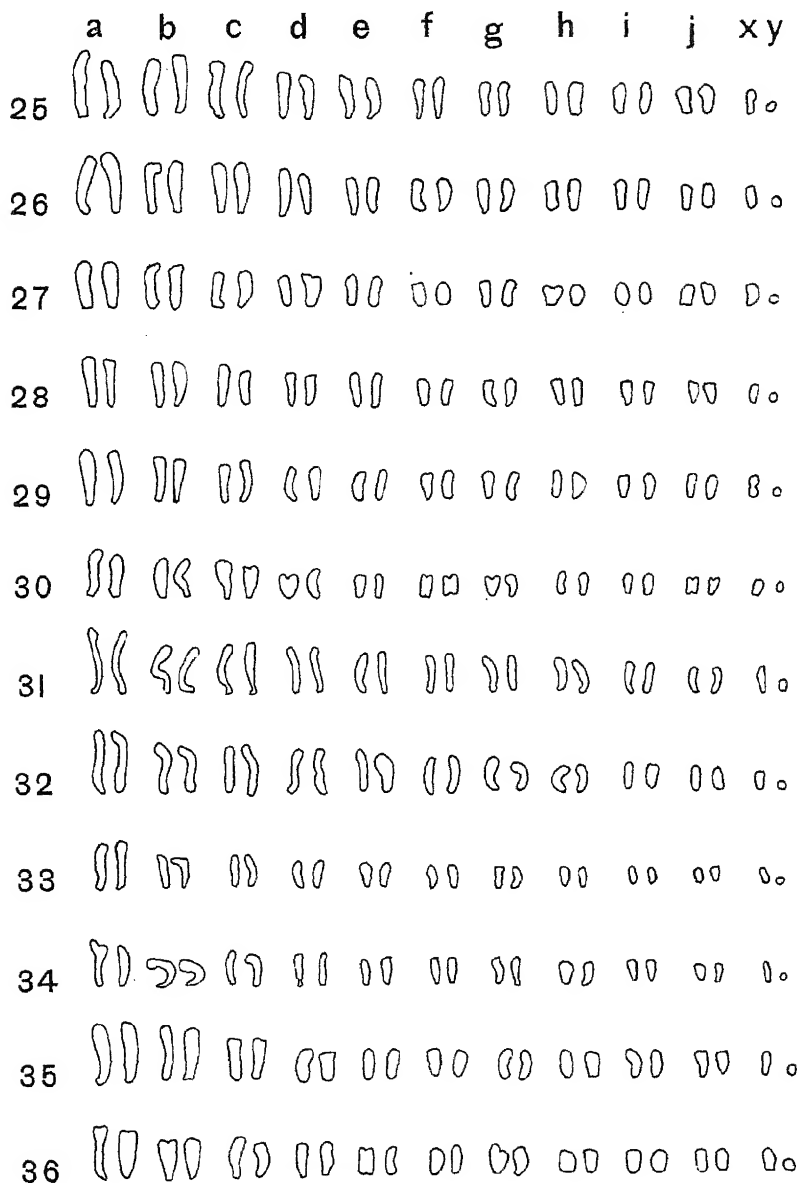


PLATE 4

EXPLANATION OF FIGURES

These figures are polar views of equatorial plates in female opossums. Figures 37 to 42 are from opossum D; figures 43 to 45 are from opossum E, and figures 46 to 48 are from opossum F.

37 From a connective-tissue cell, cortex of the kidney. Figure 3 was drawn from this.

38 From a connective-tissue cell, cortex of the kidney.

39 From a connective-tissue cell, cortex of the kidney. Figure 2 was drawn from this.

40 From a connective-tissue cell of the intestine. Figure 6 was drawn from this.

41 From a connective-tissue cell of the intestine. Figure 7 was drawn from this.

42 From a cell in the cortex of the cerebrum. Figure 12 was drawn from this.

43 From an epithelial cell of a renal tubule. Figure 13 was drawn from this.

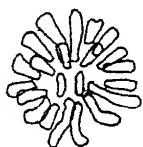
44 From a connective-tissue cell, cortex of the kidney. Figure 14 was drawn from this.

45 From a cell of the lung. Figure 15 was drawn from this.

46 From an epithelial cell of a renal tubule. Figure 19 was drawn from this.

47 From an epithelial cell of an intestinal gland. Figure 22 was drawn from this.

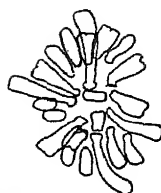
48 From a connective-tissue cell of the intestine. Figure 24 was drawn from this.



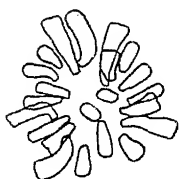
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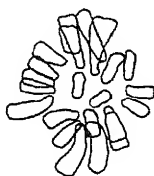
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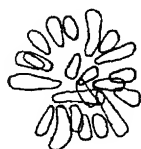
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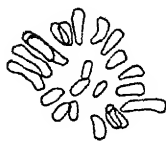
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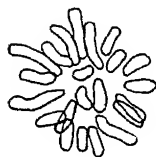
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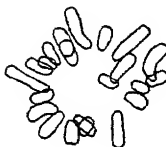
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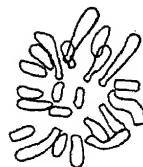
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PLATE 5

EXPLANATION OF FIGURES

All drawings on this plate represent polar views of equatorial plates from opossum G, a male. All tissues were fixed in Bouin's, dehydrated and cleared in absolute and xylol.

49 From a connective-tissue cell, cortex of the kidney. Figure 26 was drawn from this.

50 and 51 From connective-tissue cells, cortex of the kidney.

52 and 53 From connective-tissue cells, cortex of the kidney.

54 From a cell in the tunica fibrosa of the kidney.

55 From a hepatic cell. Figure 28 was drawn from this.

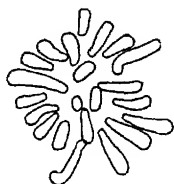
56 From a pancreatic cell. Figure 29 was drawn from this.

57 From a pancreatic cell.

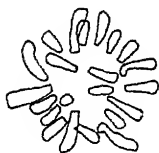
58 From a cell in the thymus.

59 From a cell in the spleen.

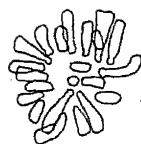
60 From an epithelial cell of the adrenal. Drawn from two sections.



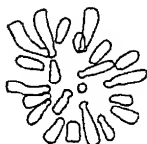
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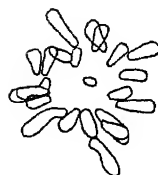
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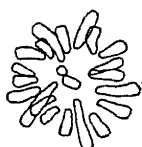
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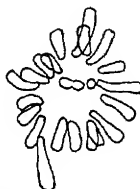
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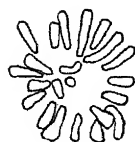
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A HISTOLOGICAL DESCRIPTION OF PIGMENT DISTRIBUTION IN THE EYES OF GUINEA-PIGS OF VARIOUS GENETIC TYPES

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FIVE PLATES (FORTY-FIVE FIGURES)

AUTHOR'S ABSTRACT

A histological study was made in the guinea-pig of the distribution of melanin pigment in the normal dark-eye and in various mutant types, such as the brown type of dark-eye (*b*), light and dark salmon-eye (*sm*), dark red-eye (*c'B*), brown red-eye (*cfb*), albino (*c'*), pink-eye (*p*), and pseudopink-eye (*P sm b*). The genes which affect pigmentation in the eye have definite qualitative or quantitative effects on the production of melanin. Eyes of the genetic constitution *c'*, *p*, or *sm* possess very little pigment. But eyes which have the dominant allelomorphs of these genes, *C*, *P*, or *Sm*, have a large amount of pigment. The non-yellow gene, *c'*, and the brown gene, *b*, cause the deeper retinal layer, which is normally intensely pigmented throughout its entirety, to become less pigmented. There is a corresponding reduction in the melanin in the pigmented areas of the iris and choroid. In all types of eyes with reduced pigment, such as the albino, pink-eye, and pseudopink-eye, pigment persists in the deeper retinal layer even when it is almost absent from other regions. Moreover, in all eyes which have less pigment than the normal type there is a decided tendency for chromatophores to be located adjacent to blood vessels. This tendency is outstanding in the iris and quite noticeable in the choroid.

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INTRODUCTION

Within the past three years our knowledge concerning the inheritance of eye colors in the guinea-pig has greatly increased. Since external examinations of the eye are not thoroughly satisfactory methods for classifying mutant types of eye colors, an attempt has been made to increase the accuracy of classification by means of a histological study of fixed, sectioned, and stained material. Furthermore, it was hoped that a study of this nature would shed light on the action and interaction of genes which cause the production of melanin pigment in the eye. This paper, then, is concerned with the morphological description of the distribution of melanin pigment in the choroid, ciliary body, retina, and iris of the normal dark-eye and most of the mutant types. The ruby-eye described by Iljin ('26) is not included in this investigation, as that material was not available.

The study described in this paper was carried on at the Bussey Institution of Harvard University under the direction of Prof. W. E. Castle, to whom I am greatly indebted for helpful suggestions and advice throughout the progress of the work.

HISTORICAL

Several distinct genetic types of eyes in the guinea-pig have been described. Most of these are distinct and without intergrading forms, but a few types recently reported tend to overlap and cause difficulty in classification.

In 1903, Castle and Allen described the mode of inheritance of albinism in the guinea-pig. Under superficial examination the albino eye seems to be devoid of pigment in the iris, choroid, and retina. The blood in the tissues of the eye causes the pink appearance. The genetic factor c^a (albinism) is solely responsible for the absence of perceptible pigment in eyes of this type.¹

¹To simplify formulae throughout this paper, the allelomorph which is expressed in the zygote will be named. If recessive, it is of course homozygous. If dominant, it may be either homozygous or heterozygous without changing the phenotype.

The red-eye, described by Wright ('15, '16), has a reddish pupil and a dark iris which may be more or less transparent. The genetic factor c^r is responsible for this condition. When the factor c^r is combined with b , the eye presents a distinct brownish-red appearance, and in this paper the type is designated 'brown red.' On the other hand, when the factor B is combined with c^r , the reddish appearance is greatly reduced, and in some cases the red gleam can be detected only in a very favorable light. As a result, this type is called 'dark red.' It was observed that a few animals of the genetic constitution $c^r B$ had eyes which were very dark in appearance and did not have a red gleam, even in the most favorable light. It is interesting to note that c^a and c^r are allelomorphic and represent the two lowest genes of the series of albino allelomorphs which is made up of C , c^k , c^d , c^r , and c^a , named in order of dominance.

The salmon-eye, described by Gregory and Ibsen ('26), is caused by the action of the gene sm and is pink in appearance, but has a dark ring of pigment, which may vary from an extremely narrow to a comparatively wide band, around the pupillary margin of the iris. For purposes of identification, the type which has an extremely narrow band is termed 'light salmon,' while the one which has a wide band of pigment is designated 'dark salmon.'

The pink-eye, caused by the genetic factor p , was reported by Professor Castle in 1912. It resembles the albino eye (c^a) in appearance; and like the albino eye, its pink color is caused by the absence of melanin pigment, and the blood imparts the pink color to the tissues of the eye. Pink-eyed guinea-pigs, designated in this paper as the 'ring-eyed pink,' have a narrow ring of pigment in the region of the ciliary body whenever the genetic factor p is combined with the factors C and B . On the other hand, when the factor p is combined with c^d and b , the animals do not have a pigmented ring in the region of the ciliary body, and are termed the 'non-ring pink.'

Dark-eye is the normal wild type found in nature, and has a black or dark iris and pupil. The genes which interact to

form this type are P (the dominant allelomorph of pink-eye, p), Sm (the dominant allelomorph of salmon-eye, sm), and C (the color factor highest in the series of albino allelomorphs). If the animal carries the genetic factor B (the gene which causes black coat color) in addition to P, Sm, and C, the eye has a black iris and pupil. This type is referred to as the 'black type of dark-eye.' If the animal carries the genetic factor b (the gene which produces chocolate coat color and is the allelomorph of B) in addition to P, Sm, and C, the eye has a dark iris and pupil which may have a reddish gleam in certain positions in the light. This type is called the 'brown type of dark-eye.' In 1909, Miss Sollas referred to the eyes of guinea-pigs with chocolate coats as 'ruby' because of their brownish-red gleam. The genetic constitution of this eye is C P Sm b.

In a paper published in *The Journal of Experimental Zoölogy*, vol. 52, no. 1, November, 1928, I described three new types of eyes which are caused by variations in amount of pigment in the salmon-eye (sm). The first, designated as 'pseudopink,' is devoid of perceptible pigment, although the gene P is present. Since the chocolate pigment in the fur of pseudopink-eyed animals is not greatly reduced, these individuals can easily be distinguished from the true pink-eyed (p) animals. The genes interacting to produce pseudopink are P, sm, and b. When the salmon-eye gene, sm, is combined with the chocolate gene, b, the pigment in the iris is reduced to an imperceptible amount.

Although all salmon-eyed (sm) animals are of the genetic constitution P sm B, two different types are evident. The light-salmon type has a narrow ring of pigment around the pupillary margin of the iris, which in extreme cases appears broken. Since the remaining portions are unpigmented, the eye appears to be pink. The dark salmon-eye has a wide, dark ring of pigment, often irregular, in the pupillary margin of the iris. A limited amount of pigment in the ciliary body and adjacent regions gives the background of the eye a dark appearance.

MATERIALS AND METHODS

The eyes used in this investigation were the black type of dark-eye, brown type of dark-eye, albino, dark red, brown red, dark salmon, light salmon, ring-eyed pink, non-ring pink, and the pseudopink. If the guinea-pig was to be used later in breeding experiments, it was etherized and the right eye removed by a method similar to that used by Keeler ('26), but if the animal was not needed for breeding, it was killed and both eyes were immediately removed. The sclera and choroid were cut with a pair of fine dissecting scissors in the region of the ciliary body so that the fixative could readily penetrate the innermost parts of the eye. Several fixatives were tried, but the most successful one was Bouin's fluid. The eye may remain in this fluid from a few weeks to several months without injury. In fact, the most successful results were obtained from eyes which had remained in Bouin's fluid from four weeks to six months.

When the eye was removed from the fixative, it was run up to 70 per cent alcohol. After this, the lens was removed through the opening previously made near the region of the ciliary body. In order to allow the lens to pass through, this opening was enlarged by cutting a small circular piece from the eye. This circular piece included a part of the sclera, cornea, ciliary body, and iris. Care had to be taken in the operation that the delicate retinal epithelial layers should not be torn loose from the iris. The lens was removed from the eye because it was of no value in the study, and when allowed to remain, it became hard in alcohol, and as a consequence the eye was exceedingly difficult to section. The eye was then run through the alcohols and cleared in xylol.

After the lens was removed, the eye had to be handled with great care that air bubbles might not penetrate the interior and get between the sclera and the choroid, or between the choroid and the retina. It was found best to grasp the cut edge of the cornea with a small pair of forceps, and to hold the cut part up, so that the liquid would remain on the inside of the eye. This would keep air out of the opening while the

phores tend to anastomose, but vary somewhat in size and are irregularly distributed. The suprachoroid contains several strata of elongated cells which are heavily pigmented and which resemble the ones found in the vascular layer.

Orbiculus ciliaris (fig. 10). The deeper retinal layer, which is a continuation of the retinal epithelium of the choroid, is richly supplied with granules of dark pigment. In the vascular area are numerous branched chromatophores resembling in structure the ones found in the posterior vascular area of the choroid. The suprachoroid contains several strata of laminated cells densely packed with granules of pigment.

Corona ciliaris (fig. 20). The deeper retinal layer of epithelium is heavily pigmented. The stroma of the ciliary process contains an occasional branched chromatophore. In the vascular area, at the base of the ciliary processes, are many branched chromatophores. The pigment cells of these three regions are similar to those found in the orbiculus ciliaris. There appear to be no pigment cells in the anterior region of the ciliary body which would correspond to those found in the suprachoroid.

Iris (fig. 29). The anterior and posterior retinal layers are densely packed with numerous granules of dark pigment. The stroma contains a large number of branched chromatophores which tend to lie in an anteroposterior direction. The anterior part of the iris beneath the epithelium has a large number of branched pigment cells which become more concentrated near the pupillary margin. The pupillary margin itself (fig. 38) is surrounded by a dense layer of pigment cells. The blood vessels of the iris are usually surrounded by chromatophores. Although in many cases the body of the chromatophore itself is not adjacent to the blood vessel, the processes from it extend around the blood vessel.

Sclera. Pigment cells are frequently found scattered in the various regions of the sclera, but apparently they have little effect on the color of the eye, since they are overshadowed by the immense amount of pigment in the choroid, iris, and ciliary body.

Brown type of dark-eye

The pigment in the brown type of dark-eye (C P Sm b) is, on the whole, brownish in color throughout the various regions. However, where it is heavily concentrated it appears blackish, but where it is thin it is of a yellowish brown color.

Choroid (fig. 9). The anterior two-thirds of the retinal epithelium is moderately supplied with granules of pigment. In the vascular layer there are numerous branched chromatophores which contain many small granules of pigment, and which tend to lie near blood vessels. Several strata of elongated pigment cells are in the suprachoroid. The pigment in the choroid appears to increase in amount as the ora serrata is approached.

Orbiculus ciliaris (fig. 18). The deeper retinal layer of epithelium contains a large number of pigment granules which become more concentrated toward the inner side. There is not enough pigment in this layer to obscure the nuclei, the outer ends of which are usually visible. The vascular area contains numerous branched pigment cells. The suprachoroid is provided with several strata of laminated chromatophores.

Corona ciliaris (fig. 28). The deeper retinal layer of epithelium is less heavily pigmented than the corresponding layer of the orbiculus ciliaris, and throughout the entire layer there is a variation in the amount of pigment. In some regions the nuclei are almost obscured by melanin; in other regions they are plainly visible. The stroma of the ciliary body contains occasional chromatophores lightly pigmented. The anterior region adjacent to the sclera contains no pigment cells which correspond to those of the suprachoroid.

Iris (fig. 34). In the region which adjoins the ciliary body the retinal layers are moderately pigmented. However, the posterior retinal layer close to the ciliary body is devoid of pigment. The stroma abounds in small branched chromatophores which seem to be more numerous toward the anterior wall and around the blood vessels. The retinal

layers of epithelium at the pupillary margin (fig. 45) have abundant granules of pigment.

Albino eye

As a general rule, under superficial examination the tissues of the albino (c^a) eye of the living animal reveal no pigment. However, when a histological study is made, a few light brownish granules of pigment are found in the retinal epithelium of the choroid (fig. 7) and in other regions of the choroid (fig. 6) which are pigmented in the dark-eye. In the orbiculus ciliaris the deeper retinal layer of epithelium contains minute granules of pigment (fig. 14). It is possible to find scattered granules of pigment in any region of the deeper retinal layer.

Dark red-eye

In the dark red-eye (c^rB) the pigment is of a brown or brownish black color. If many granules are densely packed together, the mass is of a brownish black color.

Choroid (fig. 2). The retinal epithelium of the choroid contains dark granules of pigment which are evenly distributed along the retinal edge, but the posterior part is devoid of pigment granules. The nuclei of the retinal epithelial layer are visible, since there is not enough pigment to cover them. In the vascular area are branched chromatophores which contain small granules of pigment. The suprachoroid contains several layers of elongated pigment cells which are less densely packed than those of the vascular area.

Orbiculus ciliaris (fig. 11). The deeper retinal layer of epithelium is completely pigmented throughout its entire area. The pigment granules are so dense that the nuclei are not visible in many places. The vascular area is supplied with many branched chromatophores. The pigment cells of the suprachoroid are laminated and elongated.

Corona ciliaris (fig. 21). The deeper retinal layer of epithelium is abundantly supplied with pigment granules. In the stroma there is an occasional branched chromatophore, but there are many branched pigment cells in the vascular

area. In the anterior region of the ciliary body there are no pigment cells which correspond to those in the suprachoroid.

Iris (fig. 30). The anterior and posterior retinal layers are both pigmented. As they are not so richly supplied with pigment granules as the normal dark-eye, the nuclei are perceptible. The part of the posterior layer immediately adjacent to the ciliary body does not contain pigment granules, but granules do appear and increase in number a short distance from the ciliary body. They reach their maximum number within a few cells and retain this number uniformly as they approach the pupillary margin of the iris. The stroma is well supplied with branched anastomosing chromatophores which tend to be arranged chiefly in an antero-posterior direction. The anterior part of the iris beneath the epithelium is supplied with numerous pigment cells in the region of the pupillary margin, while the pupillary margin itself is richly supplied (fig. 39). In the region of the sphincter muscle the number of pigment cells is markedly reduced. There is a decided tendency for chromatophores to be grouped about the blood vessels in the iris.

Brown red-eye

The granules in the brown red-eye ($c^r b$) are of a dark brownish color, and are less heavily concentrated than those in the dark red-eye ($c^r B$). When the granules are tenuous, they appear yellowish brown.

Choroid (fig. 3). The number of pigment granules in the retinal epithelium of the brown red-eye appears to be less than in the corresponding region of the dark red-eye. The chromatophores in the vascular area are smaller and less branched, but they are more conspicuous and more numerous near blood vessels. The chromatophores of the suprachoroid are greatly reduced and have very fine granules of pigment.

Orbiculus ciliaris (fig. 12). The deeper retinal layer of epithelium is densely packed with granules of pigment, but the outer part of the nuclei is clearly visible. The chromato-

phores in the vascular area are small and are filled with minute granules. The suprachoroid is slightly pigmented.

Corona ciliaris (fig. 22). The deeper retinal layer is lightly pigmented. The stroma has a few pigmented cells. The vascular area also contains a small number of chromatophores. The anterior region of the ciliary body adjacent to the sclera is, for the most part, devoid of pigment.

Iris (fig. 31). The anterior and posterior retinal layers are pigmented in a manner similar to the corresponding areas of the dark red-eye (c^rB). However, the granules appear smaller and less concentrated. The stroma has small pigment cells with very delicate branches. The anterior part of the iris beneath the epithelium is less heavily pigmented than the corresponding region of the dark red-eye. The retinal layers of epithelium around the pupillary margin (fig. 40) are slightly pigmented.

Dark salmon-eye

The pigment of the dark salmon-eye (P sm B) is black when arranged in thick layers, but when it is tenuous it appears somewhat brownish black.

Choroid (fig. 4). The retinal epithelium contains occasional areas of minute granules of pigment which are located near its inner wall. The vascular area contains a restricted number of cells with diffuse pigment. The suprachoroid is also provided with a limited number of scattered granules.

Orbiculus ciliaris (fig. 13). The deeper retinal layer of epithelium has a few granules of pigment which vary in size and are concentrated in the cytoplasm around the nuclei. In the vascular area there are several cells which contain diffuse granules of pigment. The cells in the suprachoroid contain a sparse amount of pigment.

Corona ciliaris (fig. 24). The deeper retinal layer of the ciliary processes contains pigment which is distributed in a diffuse manner. The stroma is supplied with a few pigment cells. In the vascular area there are numerous branched chromatophores which have scattered pigment granules. The anterior region adjacent to the sclera has no pigment.

Iris (fig. 35). The cells of the anterior and posterior retinal layers contain very little pigment, the posterior layer containing a smaller amount than the anterior. The stroma near its attachment to the ciliary body contains a large number of chromatophores. The anterior part of the iris toward the pupillary margin is supplied with numerous large branched chromatophores. At the pupillary margin (fig. 42), there is very little pigment. Chromatophores tend to be grouped around the blood vessels.

Light salmon-eye

The pigment in the light salmon-eye (P sm B) is black or brownish black.

Choroid (fig. 5). The retinal epithelium of the choroid contains very little perceptible pigment, and the amount which is present is associated, for the most part, with the anterior wall. The vascular area contains a few minute granules of pigment which are in close proximity to the blood vessels. The suprachoroid is supplied with a limited number of granules of pigment only in disconnected areas.

Orbiculus ciliaris (fig. 15). The deeper retinal layer of epithelium contains small amounts of granules of pigment scattered throughout the cytoplasm of the cell. Some of the cells in the vascular area have a few granules of pigment; the suprachoroid, likewise, contains only a small amount.

Corona ciliaris (fig. 25). The deeper retinal layer of epithelium contains a limited number of pigment granules. In general, the stroma is not pigmented, but occasional granules are found. The vascular area has a limited number of chromatophores with widely dispersed pigment granules. In the anterior region which adjoins the sclera, there are, as a rule, no pigment cells.

Iris (fig. 37). The posterior retinal layer contains no perceptible granules of pigment and the anterior retinal layer has only a restricted number. The stroma is scantily supplied with delicate chains of melanin granules. In the anterior part of the iris, toward the pupillary margin, there are

many chromatophores which contain a small amount of pigment; moreover, the pupillary margin itself (fig. 41) has only a small number. As in many of the other types of eyes, chromatophores tend to be grouped near the blood vessels of the iris.

Ring-eyed pink

The pigment granules of the ring-eyed pink (p C B) type are of a yellowish brown color.

Choroid. The retinal epithelium contains a limited number of granules which are associated with the inner wall. In the posterior region of the ora serrata these granules are more heavily concentrated. There are no perceptible granules in the vascular area. The suprachoroid has what appears to be a small number of minute granules which are faintly pigmented.

Orbiculus ciliaris (fig. 16). The cells of the deeper retinal layer have appreciable quantities of pigment. There are traces of minute pigment granules in some of the cells of the vascular area and of the suprachoroid.

Corona ciliaris (fig. 26). In the deeper retinal layer of epithelium of the ciliary processes, the pigment is distributed in greater amounts than in the corresponding epithelium of the orbiculus ciliaris. The stroma in many cases may possess isolated granules of pigment. Only a few cells of the vascular area contain pigment, and then only in almost imperceptible amounts. The region adjacent to the sclera appears to be unpigmented.

Iris (fig. 32). The anterior retinal layer contains melanin granules which are grouped around the nuclei. The posterior retinal layer is supplied with occasional granules, and the stroma has small isolated groups. In the anterior part of the iris there does not appear to be a marked amount of pigment, and in the pupillary region the small amount present is confined to the retinal layers. In this type of eye the pigment granules do not seem to clump about the blood vessels as they do in other types of eyes, but are almost exclusively confined to the epithelial layers, of which the deeper is the most densely pigmented.

Non-ring pink

The pigment in the non-ring pink (p c^d b) eye is of a yellowish brown color.

Choroid (fig. 8). The retinal epithelium is greatly deficient in pigment; however, in the region posterior to the ora serrata pigment granules are occasionally found. The vascular area and the suprachoroid, in most cases, seem to be devoid of pigment.

Orbiculus ciliaris (fig. 19). The deeper retinal layer of epithelium contains a limited number of scattered granules, but the vascular area and suprachoroid do not contain a perceptible amount.

Corona ciliaris (fig. 27). The deeper retinal layer also contains a limited number of scattered granules; likewise, in the vascular area there are traces of minute granules. The stroma and the anterior region adjacent to the sclera are unpigmented.

Iris (fig. 33). Very little pigment is found in the posterior or anterior retinal layers. The stroma adjacent to the retinal layers contains only traces.

Pseudopink-eye

The pigment in the pseudopink-eye (P sm b) is brownish in color.

Choroid. The retinal epithelium has occasional areas of granules which increase in number toward the ora serrata. There are some traces of pigment in the posterior region of the vascular area. A few regions of the suprachoroid have an infinitely small amount.

Orbiculus ciliaris (fig. 17). The deeper retinal layer of epithelium shows a small amount of pigment, but there is none perceptible in the other areas of this region.

Corona ciliaris (fig. 23). The amount of pigment varies greatly in different regions of the ciliary body. The deeper retinal layer shows minute granules in the cytoplasm. Occasional granules are visible in the stroma, but the vascular area and the region adjacent to the choroid appear to be entirely devoid of pigment.

Iris (fig. 36). In the iris, near its attachment to the ciliary body, small quantities of pigment granules are plainly visible in the anterior retinal layer. On account of their extremely small size, the granules which are present in the posterior retinal layers are scarcely visible. In the stroma small amounts of pigment occur, and near the pupillary margin (figs. 43 and 44) pigment may be found in the retinal layers. The stroma and anterior part of the iris are, for the most part, unpigmented. The pigment granules tend to be grouped near blood vessels.

DISCUSSION

In the study of the inheritance of some of the mutant-eye types found in the guinea-pig, living eyes were examined and classified. After a study of the distribution of pigment was made from sectioned material, it was found that there is a high correlation between the amount of pigment which actually exists as shown by a histological study and the amount which appears to exist from external examination. It was also observed that from an examination of the living eye one could ascertain with a moderate degree of certainty the exact areas of the eye which would be found pigmented when the material was sectioned. Moreover, the eyes may be divided into two classes, according to the amount of pigment which they contain. All animals of the genetic constitution c^a , p , or sm have very little pigment in the eye, but all other animals, which carry the dominant allelomorphs C , P , or Sm , have heavily pigmented eyes.

Living ring-eyed pink (pBC) eyes have a ring of dark pigment which appears distinctly on the outer periphery of the iris. When histological sections of these eyes are examined, it is found that in the region of the corona ciliaris the deeper retinal layer of epithelium contains appreciable amounts of melanin granules. Although the living non-ring pink (pbc^a or c^r) eye does not appear to have a ring of pigment on the outer periphery of the iris, a histological examination shows that faint traces of pigment actually exist

in the deeper retinal layer of the corona ciliaris (compare fig. 26 of the ring-eyed pink with fig. 27 of the non-ring pink).

From a study of the living salmon-eye (sm), either light or dark, it is easy to predict the exact regions in which the pigment will be found most highly concentrated. In the living eye of this type only the pupillary margin of the iris appears to contain pigment, and in sectioned material this region alone shows appreciable amounts. Furthermore, the band of pigment around the pupillary margin is comparatively wide in the living dark salmon-eye, and sectioned preparations show a large amount of pigment in this region. In both types of salmon-eye there may be occasional chromatophores and melanin granules in all the regions which are normally pigmented; however, in most individuals only the pupillary margin of the iris reveals perceptible pigment in the living eye. In some dark salmon-eyed animals pigment may show through the cornea from the deeper retinal layer of the corona ciliaris.

Although the albino (c^a) eye and most pseudopink (P sm b) eyes in the living condition show no pigment, histological sections always reveal slight amounts of pigment which are usually associated with the deeper retinal layer of epithelium. Moreover, in the albino eye the most deeply pigmented area is in the region of the ciliary processes. On the other hand, the iris at the pupillary margin is the most heavily pigmented region in the pseudopink-eye, although even here the pigment is very sparse in most individuals. In fact, the pigment is greatly reduced in the pseudopink-eye, which is presumably of the light-salmon type. In the pseudopink and albino types the pigment granules in the retinal layer in the region of the choroid are probably associated with the visual rods as they appear between the retinal epithelial layer and the rods of the retina. However, the salmon-eye types may possess pigment in the retinal epithelial layer of the choroid which is not distinctly associated with the rods.

In normal dark-eyed (CP Sm B) animals all regions of the eye are deeply pigmented. The deeper retinal layer is

extremely pigmented throughout its entirety, but the superficial retinal layer is pigmented chiefly in the iris. The stroma of the iris contains many branched anastomosing chromatophores. In all parts of the choroid numerous branched chromatophores which contain an immense amount of pigment give the fixed choroid the appearance of a very dark continuous sheet.

In 1916, Wright showed that the series of albino allelomorphs affect the density of the coat color, and that some of the genes of this series affect eye color; for instance, c^r , which stands above albinism (c^a) in the series, causes a reddish gleam in the eye. When histological preparations of eyes of c^r black animals are examined, it is found that this type of eye is less pigmented in all its regions than eyes of C animals. Furthermore, the deeper retinal layer is densely pigmented throughout, and chromatophores in all parts of the eye appear quite as numerous as in normal C eyes; however, the pigment granules appear less dense in color and are less concentrated. Living c^d and c^k eyes appear dark and no reduction in pigment can be detected by observations. Since eyes of the c^d and c^k type were not studied histologically, only conjectures can be made concerning the morphological distribution of their pigment. A high correlation exists between the pigment in the hair and in the eyes of all animals, and from this fact it is reasonable to assume that quantitative differences exist between the pigments of c^d , c^k , and C eyes. Moreover, animals of the genetic constitution c^d , then, should have less pigment in the eye than the c^k and C types. Since the black fur of c^k animals is not materially diluted, one would expect only a very slight quantitative difference between the pigment in the eyes of c^k and C animals. As it is assumed that slight differences in pigmentation do exist in the eyes of c^d , c^k , and C animals, the question arises as to why one is unable to detect these differences in the living eye. The most logical explanation seems to be that all three types contain an exceedingly dense continuous pigmented layer, and, as a result, minor differences in the living eye cannot be

detected. However, a careful study of histological preparations would probably reveal the slight differences which very likely exist.

The gene *B* and its allelomorph, *b*, modify the pigment in the eye to such an extent that the change can be observed in the living eye. The eyes of animals with chocolate (*Cb*) fur are brown or brownish, and are called 'ruby' by some investigators. This color is caused by a difference in the hue of pigment. As a rule, animals of the genetic constitution *Cb* have a reddish gleam in the iris, and histological preparations of *Cb* eyes show a reduction of pigment, in quality and quantity, from the normal *CB* dark-eye. On the other hand, individuals of the genetic constitution *c^rb* always show a distinct reddish gleam through the iris, the genes *c^r* and *b* both operating together to reduce the pigment. When sectioned eyes of this type are studied, it is observed that there is a marked reduction in the quality and quantity of pigment in all parts of the eye. Moreover, the pigment in the iris does not appear to form a continuous screen, and, as a result, light may filter through the iris to the deeper parts of the eye. Iljin ('26) suggested that the reddish gleam or iridescence of eyes with reduced pigment is caused by numerous breaks in the iris which admit light to the retina. This is unmistakably the case in the *c^rb* eye, and the iridescence of the *c^rB* type evidently can be accounted for in a like manner, although the *c^rB* eye has more pigment in its iris. It was previously stated that some *c^rB* animals have been observed in which the eyes did not show a reddish gleam, but were completely dark. It is assumed that in these animals the pigment of the iris forms an unbroken screen and does not admit light to the retina.

One interesting observation which merits special attention is that chromatophores tend to cluster around the blood vessels of the eye. In eyes normally pigmented this tendency is not striking, since the pigmented cells are heavily distributed in all regions. In eyes which have a reduced amount of pigment the chromatophores tend to be concentrated

around the blood vessels, and in the salmon type the chromatophores are almost exclusively centered around capillaries. Loeb ('93) observed in his study of the chromatophores in the yolk sac of *Fundulus* that pigment cells migrate to blood vessels. In the case of the eyes of the guinea-pig it has not been ascertained whether the chromatophores migrate to blood vessels or whether they are originally formed there. Since oxidation processes seem necessary for pigment production, the chromatophores around blood vessels may be in the most favorable region to fulfill their potential capacity for pigment production.

Many investigators in biochemistry have shown that the physiology of pigment production is a complex process involving a chromogen base which is oxidized by various ferments to form pigments. It is not the purpose of this paper to discuss the physiological production of pigments, but it should be pointed out that the genes which affect pigment in the eye appear to be specific in their action, and the eyes described may represent types in which the pigment bases are in various states of oxidation. It may be possible to increase the amount of pigment in some of the types of eyes which have a reduced amount of pigment by treating them with proper ferments. If this is possible, the morphogenesis of pigment production could be attacked experimentally, and more light might be shed on the action of genes in the production of pigment.

SUMMARY AND CONCLUSIONS

1. A histological study was made of the distribution of pigment in the normal dark-eye (C P Sm B), brown-eye (b), light and dark salmon-eye (sm), red-eye (c^r), albino (c^a), pink-eye (p), and pseudopink-eye (P sm b) of the guinea-pig. Favorable sectioned material was made from these eyes by removing the lens and using ordinary histological methods of technique.

2. The appearance of the density and location of pigment in the living eye gives an excellent indication of the actual

density and distribution as determined by a histological study of the sectioned eye.

3. All the genetic types of eyes studied reveal pigment in prepared sections. The albino (c^a) was found to contain the least amount of pigment, and certain types of the pseudopink-eye ($P\ sm\ b$), presumably those of the light-salmon type, have only slightly more pigment than the albino. Pink-eyed animals of the genetic constitution CB have an appreciable amount of pigment in the deeper retinal layer in the region of the orbiculus ciliaris which causes the living eye to have a perceptible dark ring in this region. When the pink-eye gene, p , is combined with b and with the albino allelomorph c^d or c^r , a ring of pigment cannot be observed in the living eye, but histological sections reveal pigment in the deeper layer of the retinal epithelium.

4. The light and dark types of salmon-eye reveal more pigment in the pupillary margin of the iris than in any other region. The deeper layer of the retinal epithelium contains slight amounts of pigment throughout its entirety. Occasional chromatophores may be found in the choroid and in the stroma of the iris. The difference between the pigmentation of the light and the dark salmon-eye is quantitative. The dark salmon-eye has a stronger tendency to be pigmented in all its regions than the light-salmon type.

5. All animals which are not albino (c^a), pink-eyed (p), or salmon-eyed (sm) have a large amount of pigment in the tissues of the eye. The wild type of dark-eye ($CP\ Sm\ B$) contains the greatest amount of pigment in all regions. Dark eyes of the genetic constitution b have brownish pigment, which appears blackish when concentrated. When the non-yellow gene, c^r , is combined with B , there is a marked reduction in the quantity of pigment in all the regions of the eye, and also a qualitative change. Individuals of the genetic constitution $c^r\ b$ possess less pigment in all regions of the eye than the $c^r\ B$ type, and the pigment screen of the iris is broken in numerous places.

6. In the wild type of dark-eye the deeper layer of the retinal epithelium is the most deeply pigmented part, and in all types of eyes with reduced melanin, pigment persists in this layer even when it is absent from other regions.

7. In eyes which have a reduced amount of pigment there is a decided tendency for the chromatophores to be located around the blood vessels.

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EXPLANATION OF PLATES

All figures on the accompanying plates were made from material fixed in Bouin's fluid, stained in Ehrlich's haematoxylin, and counterstained with eosin. The figures were outlined with the aid of a camera lucida at a magnification of 1325 diameters unless otherwise stated. Cell walls are indicated by stippled lines. Pigment is represented by stippled masses. In the reproduction the figures were reduced one-half.

ABBREVIATIONS

<i>AE</i> , anterior epithelium	<i>PR</i> , posterior retinal layer
<i>AR</i> , anterior retinal layer	<i>RE</i> , retinal epithelium of the choroid
<i>B</i> , blood vessels	<i>S</i> , suprachoroid
<i>C</i> , chromatophore	<i>SR</i> , superficial retinal layer
<i>DR</i> , deeper retinal layer	<i>St</i> , stroma
<i>P</i> , pigment granules	<i>V</i> , vascular area
<i>PM</i> , pupillary margin of the iris	

PLATE 1

EXPLANATION OF FIGURES

Sections of the choroid of various types of guinea-pigs as indicated.

1 Black type of dark-eye; genetic constitution, P Sm CB. Normal wild type of eye. Retinal epithelium of choroid is intensely packed with dark granules of pigment. Pigment cells in vascular area are packed with dark granules. Chromatophores of suprachoroid are heavily pigmented.

2 Dark red-eye; genetic constitution, P Sm c^r B. Inner part of retinal epithelium of choroid is heavily pigmented; outer part is sparsely pigmented. Pigment cells in vascular area are moderately pigmented; those in the suprachoroid are also moderately pigmented.

3 Brown red-eye; genetic constitution, P Sm c^r b. Inner part of retinal epithelium of choroid contains a small number of brownish granules of pigment. The outer part has no pigment. Pigment cells in vascular area are few and contain brownish granules. Suprachoroid has only scattered pigment cells, and these contain small amounts of brownish-colored pigment.

4 Dark salmon-eye; genetic constitution, P sm CB. Retinal epithelium of choroid is devoid of pigment. Chromatophores in vascular area and in suprachoroid are few.

5 Light salmon-eye; genetic constitution, P sm CB. Retinal epithelium of choroid is devoid of pigment. In the vascular area the cells are thinly scattered, and these contain only a few granules of pigment.

6 Albino eye; genetic constitution, c^a. A few cells in the vascular area contain a limited number of pigment granules. Suprachoroid has no pigment granules.

7 Albino eye; genetic constitution, c^a. Retinal epithelium of choroid contains minute granules of pigment.

8 Non-ring pink-eye; genetic constitution, p c^a b. The retinal epithelium of the choroid, vascular area, and suprachoroid is devoid of pigment.

9 Brown type of dark-eye; genetic constitution, P Sm Cb. Inner part of retinal epithelium of choroid is pigmented. Lightly pigmented chromatophores are in the vascular area and in the suprachoroid.

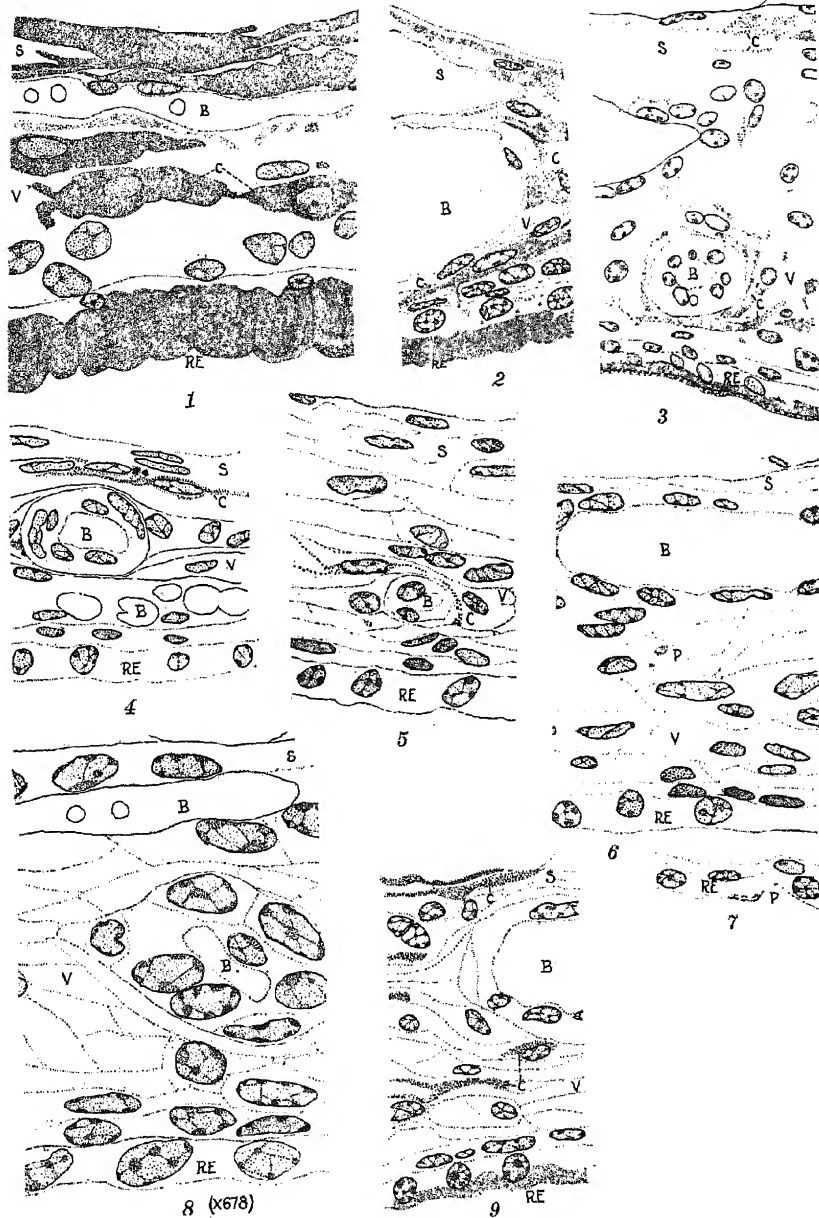


PLATE 2

EXPLANATION OF FIGURES

Segments of sections through the orbiculus ciliaris of various types of guinea-pigs as indicated.

10 Black type of dark-eye; genetic constitution, P Sm CB. Deeper retinal layer, vascular area, and suprachoroid contain an exceedingly large amount of pigment. Superficial retinal layer is unpigmented.

11 Dark red-eye; genetic constitution, P Sm c^r B. Deeper retinal layer, vascular area, and suprachoroid are rather thickly pigmented.

12 Brown red-eye; genetic constitution, P Sm c^r b. Deeper retinal layer, vascular area, and suprachoroid are moderately pigmented.

13 Dark salmon-eye; genetic constitution, P sm CB. Deeper retinal layer has a light amount of pigment closely associated with the nuclei. Vascular area and suprachoroid have small areas which contain a limited number of pigment granules.

14 Albino eye; genetic constitution, c^a. Deeper retinal layer contains a few diffuse pigment granules. Vascular area and suprachoroid contain no pigment.

15 Light salmon-eye; genetic constitution, P sm CB. Deeper retinal layer has scattered areas of pigment. Vascular area and suprachoroid have a very few pigment granules.

16 Ring-eyed pink-eye; genetic constitution, p CB. Deeper retinal layer is moderately pigmented around the nuclei.

17 Pseudopink-eye; genetic constitution, P sm C b. Deeper retinal layer is slightly pigmented. Granules are concentrated around the nuclei. Vascular area and suprachoroid contain no pigment.

18 Brown type of dark-eye; genetic constitution, P Sm C b. Deeper retinal layer contains much pigment which obscures the outer ends of the nuclei.

19 Non-ring pink-eye; genetic constitution, p c^d b. Deeper retinal layer is very lightly pigmented.

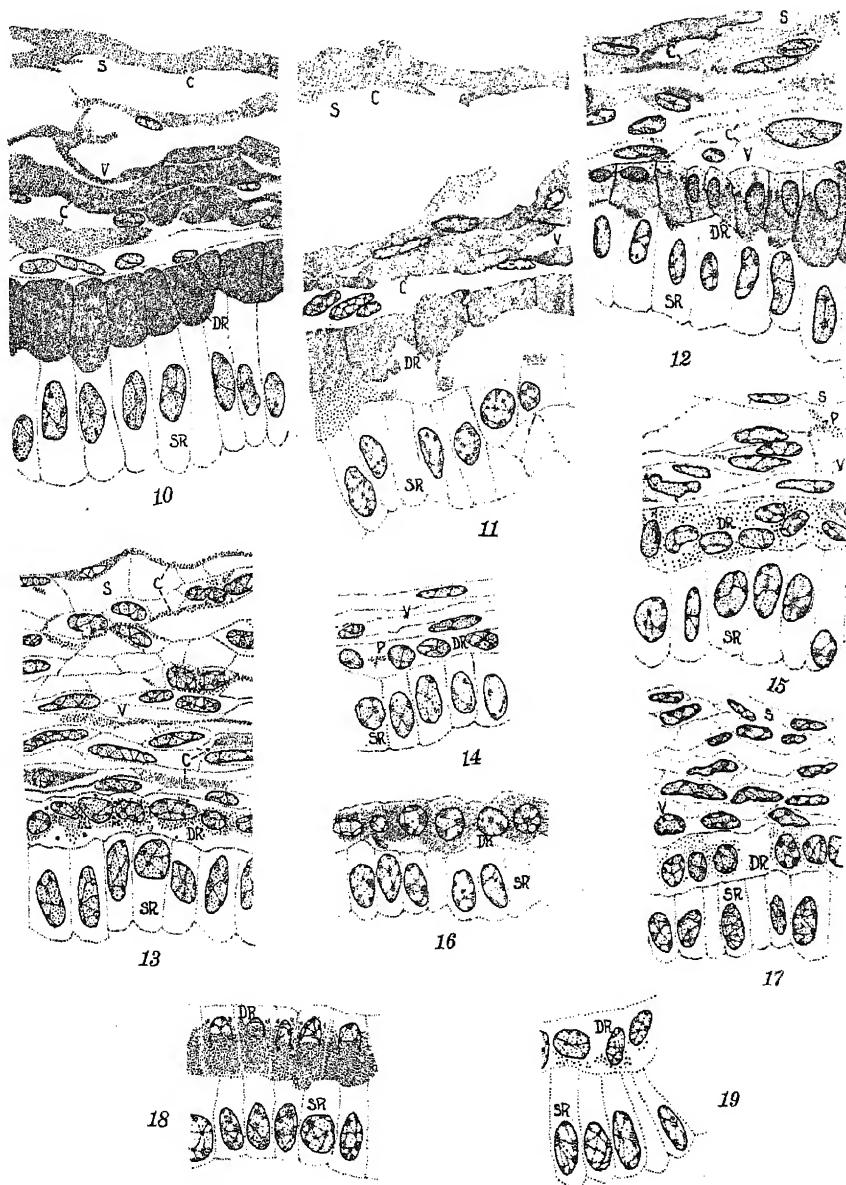


PLATE 3

EXPLANATION OF FIGURES

Sections through the corona ciliaris of various types of guinea-pigs as indicated, showing the retinal epithelial layers on one side only.

20 Black type of dark-eye; genetic constitution, P Sm C B. Deeper retinal layer and a few cells in the vascular area contain an exceedingly large amount of pigment.

21 Dark red-eye; genetic constitution, P Sm c^r B. Deeper retinal layer and a few cells in vascular area are thickly pigmented.

22 Brown red-eye; genetic constitution, P Sm c^r b. Deeper retinal layer and a few cells in vascular area are moderately pigmented.

23 Pseudopink-eye; genetic constitution, P sm C b. Deeper retinal layer lightly pigmented, with granules grouped about the nuclei. Cells in vascular area do not contain pigment.

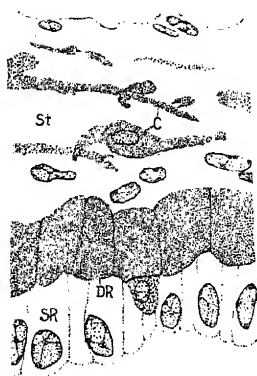
24 Dark salmon-eye; genetic constitution, P sm C B. Deeper retinal layer contains numerous granules of pigment. Cells in the vascular area contain no perceptible pigment granules.

25 Light salmon-eye; genetic constitution, P sm C B. Deeper retinal layer has scattered areas of pigment. Cells in the vascular area contain no pigment.

26 Ring-eyed pink-eye; genetic constitution, p C B. Deeper retinal layer is moderately pigmented.

27 Non-ring pink-eye; genetic constitution, p c^d b. Deeper retinal layer has diffuse pigment granules closely associated with the nuclei.

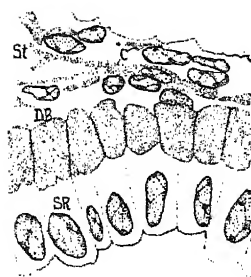
28 Brown type of dark-eye; genetic constitution, P Sm C b. Deeper retinal layer is heavily pigmented. Vascular area has no pigment.



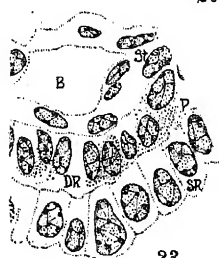
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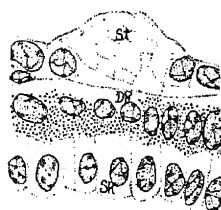
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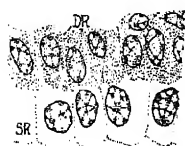
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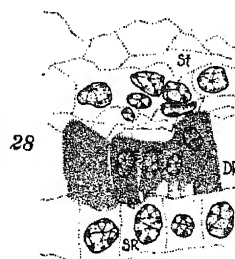
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PLATE 4

EXPLANATION OF FIGURES

Sections through the iris, near its attachment to the choroid, of various types of guinea-pigs as indicated.

29 Black type of dark-eye; genetic constitution, P Sm C B. Anterior and posterior retinal layers are heavily packed with dark granules of pigment. Stroma has many branched anastomosing chromatophores.

30 Dark red-eye; genetic constitution, P Sm c^r B. Anterior and posterior retinal layers have only a moderate number of pigment granules. The stroma has many branched anastomosing chromatophores.

31 Brown red-eye; genetic constitution, P Sm c^r b. Anterior and posterior retinal layers have a reduced number of brownish granules of pigment, the anterior retinal layer being the more heavily pigmented. The stroma has many cells which contain a reduced number of pigment granules.

32 Ring-eyed pink-eye; genetic constitution, p B C. Posterior retinal layer contains no pigment. Anterior retinal layer has a greatly reduced amount of pigment.

33 Non-ring pink-eye; genetic constitution, p c^d b. Posterior retinal layer contains no pigment. Anterior retinal layer shows only a small number of pigment granules, and these are closely associated with the nuclei.

34 Brown type of dark-eye; genetic constitution, P Sm C b. Anterior and posterior retinal layers are moderately pigmented. Many branched anastomosing chromatophores are found in the stroma. Anterior wall of iris is pigmented.

35 Dark salmon-eye; genetic constitution, P sm C B. Posterior and anterior retinal layers contain only a few scattered granules of pigment. Stroma contains numerous chromatophores which have a moderate amount of pigment.

36 Pseudopink-eye; genetic constitution, P sm C b. Posterior retinal layer has no pigment granules. Anterior retinal layer contains a few scattered areas of loosely grouped pigment granules. Stroma has a limited number of nuclei which have a few pigment granules loosely grouped about them.

37 Light salmon-eye; genetic constitution, P sm B. Posterior retinal layer is devoid of pigment. Anterior retinal layer has only a few isolated groups of pigment. Stroma contains a limited number of chromatophores which have delicate chains of pigment granules.

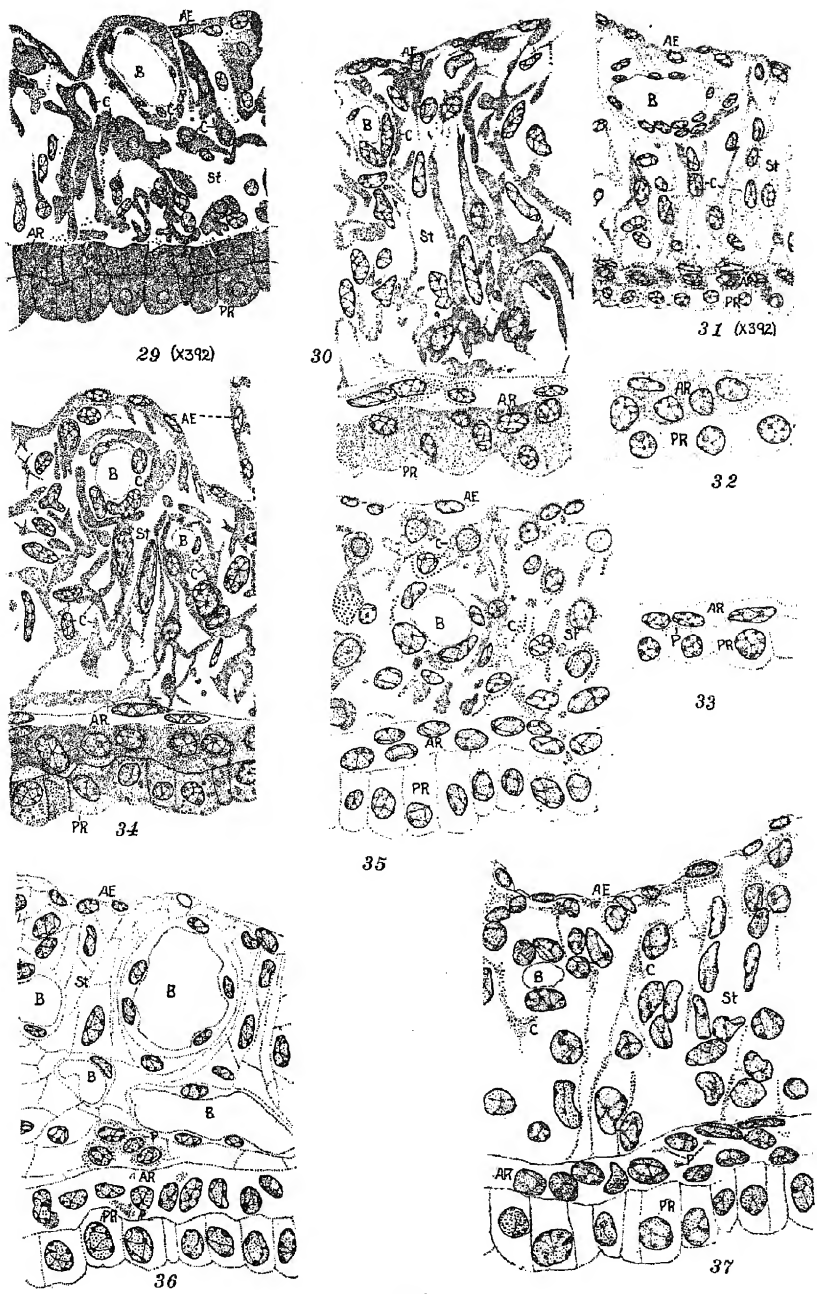


PLATE 5

EXPLANATION OF FIGURES

Sections through the pupillary margin of the iris of various types of guinea-pigs as indicated.

38 Black type of dark-eye; genetic constitution, P Sm CB. Retinal layers are densely packed with pigment granules. Stroma has a limited number of chromatophores.

39 Dark red-eye; genetic constitution, P Sm c^r B. Retinal layers are moderately pigmented. Stroma contains only a few pigment granules. Anterior part of iris contains a moderate amount of pigment granules.

40 Brown red-eye; genetic constitution, P Sm c^r b. Both retinal layers are lightly pigmented. Stroma contains only a few cells of pigment granules.

41 Light salmon-eye; genetic constitution, P sm B. Retinal layers contain no pigment granules. Stroma has a very few chromatophores toward the anterior surface. Anterior epithelium and the part of the iris adjacent to the pupil contain a few pigment granules.

42 Dark salmon-eye; genetic constitution, P sm CB. Stroma adjacent to the pupil contains several branched chromatophores which are rather densely packed with pigment granules. The anterior epithelium of the iris contains a few pigment granules.

43 Pseudopink-eye; genetic constitution, P sm Cb. Part of the iris adjacent to the pupillary margin contains a few pigment granules.

44 Pseudopink-eye; genetic constitution, P sm Cb. Both retinal layers have a few pigment granules.

45 Brown type of dark-eye; genetic constitution, P Sm Cb. Both retinal layers are heavily pigmented. Stroma contains a limited number of chromatophores.



CYTOLOGICAL STUDIES ON THE SPINNING GLANDS OF PLATYPHYLAX DESIGNATUS WALKER (TRICHOPTERA): RESPECTIVE RÔLES PLAYED BY THE NUCLEUS AND THE GOLGI APPARATUS DURING SECRETION

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THREE PLATES (FOURTEEN FIGURES)

AUTHORS' ABSTRACT

In sections the protoplasm of the spinning gland of *Platypylax designatus* Walker appears to be a syncytium with large, branching nuclei, which contain both nucleoli and chromatin granules embedded in a delicate linin reticulum.

In the normal gland the nucleus is sharply marked off by a nuclear membrane from the granular, homogeneous cytoplasm. The Golgi apparatus appears as rings, loops, or comma-like structures, evenly distributed and without orientation to the nucleus or lumen of the gland.

The nucleoli first increase both in number and size within the nucleus, then migrate out into the cytoplasm, where they undergo further growth, and finally are dissolved and passed out into the lumen of the gland as liquid secretion.

Small vacuoles appear near the periphery of the gland in early periods of activity.

In glands active for forty-eight hours all stages of the secretory process may be seen.

In glands active for longer periods only vacuoles are present which we interpret as the remains of the secretory inclusions. There is a progressive decrease in the nuclear content of the nucleus.

Throughout the activity of the gland the Golgi apparatus changes little from the normal condition, both in form and distribution. As activity progresses, the bodies become smaller and slightly more dispersed. No relation of Golgi apparatus to the secretory phenomenon is apparent.

INTRODUCTION

The large amoeboid nuclei in the silk glands of certain insects have attracted the attention of previous investigators of the anatomy and physiology of these organs, but, as far as we are aware, with the possible exception of the fragmentary work of Miss Kinney ('26), no study of the Golgi apparatus of these glands has been reported.

Marshall and Vorhies ('06) made a rather extensive study of the microscopical anatomy of the spinning glands of *Platypylax* larvae during their various periods of physiological activity. These two investigators suggested in their drawings that the nucleus might play an important part in the

metabolism of the cell. It was left, however, for Maziarski ('11) to demonstrate definitely that certain of the nucleoli migrate from their normal position into the cytoplasm of the cell, forming the material for secretion. More recently, Nakahara ('17), working on the larvae of certain of the cabbage butterflies and caddis flies, concluded that "in the silk glands of insects studied, a portion of the nucleoli migrates into the cell body, and it forms at least a part of the secretion products of the cell."

Miss Kinney ('26), using the silk glands of the larvae of *Hyphantria cunea*, described three types of bodies in the nucleus: chromatin, nucleoli, and nucleoloid bodies. The nucleoloid bodies appear to constrict off parts of themselves, and these increase in size for a time within the nucleus, but later pass through the nuclear membrane into the cytoplasm of the cell, where they undergo further modification, and are finally discharged into the lumen of the gland. Miss Kinney likewise described the appearance of mitochondria in the cells of the spinning glands: these she found as filamentous, rod-shaped, or granular structures which always showed a characteristic orientation in relation to the lumen of the gland. Their exact function in secretion was not clear. She reported that no definite structure could be found that would represent the true Golgi apparatus, although following the Golgi technique there appeared at the periphery of the cell numerous granules which, she suggested, might be fatty granules or by-products of the cell.

Noel and Paillot ('27) showed that in the sericigenous glands of *Bombyx* there exist two types of structures in the nucleus: the chromatin granules which take on a basic stain and the plasmosomes which take on an acidophile stain. They described the migration of the acidophile bodies into the cytosome, where these bodies gradually take up the basic stain and later are carried away by the streaming of the cytoplasm. They believe the new plasmosomes to be formed from the chromatin material.

The problem of the relationship of the Golgi bodies to the secretory process dates from their first description in gland cells by Negri ('99). More recently, our knowledge of their relationship to the secretory phenomenon has been extended by the ingenious researches of Nassonov ('23, '24), Bowen ('23, '24, '26), Ludford ('25), and others. In addition to the fact that a close topographical relationship exists between the Golgi apparatus and the secretory granules, Nasonov ('23) and Bowen ('24, '26) have brought forth evidence to show that these granules are actually products of the Golgi material.

We wish to take this opportunity to express our sincere appreciation to Prof. M. F. Guyer for his advice and criticism in connection with this study. We wish, also, to acknowledge our indebtedness to Prof. W. S. Marshall for his kind suggestions, criticisms, and advice during the course of this work. He has kindly permitted us the privilege of seeing his slides used in connection with his studies on the spinning glands of *Platyphylax* larva (Marshall and Vorhies, '06) and the use of his complete library; for all of which we are very thankful. Finally, we wish to express our thanks to Miss Hattie J. Wakeman for making figures 5 and 6, Mr. R. K. Meyer for suggestions in photography, and Mr. P. S. Henshaw for reading the manuscript.

PURPOSE

This work on the spinning glands of the trichopterous larva was undertaken with three primary studies in view: first, to ascertain if the secretory substance of the spinning glands of the trichopterous larva is derived directly from the nucleus; secondly, to determine if there is a true Golgi apparatus present in the spinning glands; and thirdly, if there is a true Golgi apparatus present, to determine its probable relationship to the phenomenon of secretion.

MATERIAL AND METHOD

The material for this study consisted of the larvae of the caddis fly, *Platyphylax designatus* Walker, which were secured in great numbers in certain of the cold springs on the southern shore of Lake Wingra, near Madison, Wisconsin. The larvae were collected and placed in an aquarium supplied with running lake water. The ease with which the larvae of *Platyphylax* can be induced to build new cases makes it possible to obtain glands of known periods of activity. The cases were removed from the larvae daily up to 360 hours of activity, at which time most of the larvae had completely stopped building new cases.

Usually the silk glands were taken out by the decapitation method and placed in the fixing fluid; however, sometimes the glands were dissected out in saline, and these also proved to be good material. The following fixing fluids were used: Bouin's, Flemming's strong, and acetic sublimate; as a whole, Flemming's gave the best fixation. The stains used included Heidenhain's hematoxylin alone and counterstained with eosin, Delafield's hematoxylin, Mallory's triple, and the triple stain of Flemming. To demonstrate the Golgi apparatus, Mann-Kopsch, Ludford's modification of Mann-Kopsch, Kolatchev's method as described by Nasonov ('23), and Da Fano's cobalt-silver-nitrate method were used. The best preparations were secured by following Ludford's modification of the Mann-Kopsch method. It was found profitable to follow the suggestion of Nasonov ('24) in removing a piece of the tissue from the 2 per cent osmic acid at various intervals, in order to determine the degree of impregnation. Da Fano's cobalt-silver-nitrate method did not prove useful with this material. The tissues were run through the ordinary paraffin method and sectioned at 5 μ .

OBSERVATIONS AND CONSIDERATIONS

The spinning glands in *Platyphylax* larva are two in number and about one and a half times the length of the body. They are folded three times as described by Gilson, Marshall

and Vorhies, and lie lateral and slightly ventral to the intestine. Each is divided into two portions, an anterior conductive and a posterior secretory portion. In sections, the glands present the appearance of a syncytium with large amoeboid nuclei embedded in a granular homogeneous cytoplasm. During the periods of activity the nuclei in the conductive portion of the gland are smaller, with a less degree of branching than those in the secretory portion. However, only a slight difference is noticeable in the nuclei of the secretory and of the conductive portions of the gland during the period of rest.

Normal gland

The cytoplasm of the normal gland (fig. 1) shows very little differentiation in the various parts of the cell; it appears dense, granular, homogeneous, stains evenly throughout all its parts, and, in practically all cases, presents an appearance free from vacuoles, migrated nucleoli, and secretory inclusions. A definite limiting membrane is present on the external as well as on the internal surface of the gland, probably comparable to the tunica propria and tunica intima of Helm ('76). The nucleus is surrounded by a distinct membrane which presents a definite demarcation between nucleus and cytoplasm. Sections of the nucleus, as Marshall and Vorhies have described, show a great variation in size and nucleolar content. The nucleoli appear to have a varied range in size and are usually found equally distributed throughout the nucleus. Some have the appearance of a fusion of two or more smaller nucleoli. They usually are more or less ovoid, although irregular surfaces are not uncommon. The chromatin granules are very much smaller than the nucleoli and are distributed fairly uniformly throughout the nucleus, as described by Marshall and Vorhies ('06). Among the earlier workers there was considerable discussion as to just what constituted the real chromatin material in the nucleus of the silk gland of insects. Korschelt ('96) insisted that the larger granules were the chromatin and the smaller ones the nucleoli,

by virtue of their staining reactions. Meves ('97), Flemming ('97), Henneguy ('04), Marshall and Vorhies ('06), and Nakahara ('22), with whom we agree, have arrived at exactly opposite conclusions. The nucleoplasm presents a very fine granular structure, having embedded within it a delicate linin reticulum, which in most cases takes on a lighter stain than do the chromatin and nucleolar bodies. Only in very successful preparations can its delicate structure be observed.

Golgi apparatus in normal gland

The Golgi apparatus in the silk glands of the caddis-fly larva impregnates with remarkable clearness (figs. 5 and 6). It is evenly distributed throughout the gland and presents a ring-, loop-, or comma-like appearance. There is no evidence of a net-like structure present or concentration of the Golgi material in any one particular area of the gland. One might have expected a definite relationship existing between the Golgi apparatus and the nucleus, but, as far as we can determine, such does not exist. Miss Kinney found no structure in the spinning glands of *Hyphantria cunea* comparable to the true Golgi apparatus. From the description of her preparations, it would seem that she was observing material which had been only lightly impregnated. We have observed similar pictures in our preparations that had remained in osmic acid for an insufficient period of time.

Active glands

The general changes in the morphology of the gland during the different periods of activity have been described with great care by Marshall and Vorhies. In the early periods only slight changes in the glands are noticeable. In the nucleus there is apparently an increase in the number of nucleoli accompanied by a slight increase in volume (fig. 8). The nucleus, in general, shows a tendency to become branched and elongated. The cytoplasm, for the most part, has lost its true homogeneous appearance and takes on a streaming

effect, which is particularly striking in the regions between adjacent nuclei. Glands after twelve hours of activity often show a number of small vacuoles along the outer border of the gland, as described by Marshall and Vorhies ('06), although their significance was not clear. It is very probable that they are directly connected with the secretory phenomenon which will be mentioned later. During this period of activity practically no change in the Golgi apparatus from the normal is apparent.

Glands active for twenty-four hours

In glands which have been active for twenty-four hours the nucleus has become enlarged and the nucleoli have increased definitely in size. In a few cases the nucleoli may be seen migrating from their normal position into the cytoplasm of the cell. It is interesting to note that the migration, for the most part, is from the outer surface of the nucleus. The nucleoli increase in number until they fill the major portion of the nucleus. The chromatin granules still appear normal. Considerable change in the cytoplasm is apparent in glands active for twenty-four hours. In the cytoplasm between the nuclei quite definite streaming-like striations are present. In some cases vacuoles may be present near the outer surface of the gland. On the inner side of the gland definite drops of secretory substance are often seen migrating into the lumen.

Only a slight change in the Golgi apparatus can be observed. The Golgi bodies appear a trifle smaller than those in the normal, but still possess their characteristic shapes. No definite connection with the secretory inclusions is obvious, although the latter, in rare cases, have associated with them smaller particles of the Golgi material.

Glands active for thirty-six hours

Figure 2 shows a gland active for thirty-six hours. The vacuoles appear to be more numerous in the peripheral area of the cell than in the gland active for a twenty-four-hour period. The striking object in this figure, however, is the

apparent heterogeneous structure of the nucleoli. The center of each of the two large, spherical nucleoli resembles, by virtue of its staining reaction, the true nucleolus of the cell, while surrounding it is a denser or darker area which has attached to its periphery numerous chromatin granules. It seems probable that the growth of some of the nucleoli within the nucleus is dependent on the chromatin granules; however, it must be pointed out that this condition is not observed in all cases, and therefore complicates the problem of the origin and growth of the nucleoli.

Glands active for forty-eight hours

Glands which have been active for forty-eight hours offer the best material for the study of the mechanism of secretion. Figure 6 shows that the nucleoli within the nucleus are of two different forms; those which have taken on a spherical or ovoid shape and those which are irregular. The ones possessing the rounded surfaces are usually the larger, and no doubt are in preparation for migration into the cell body. The outer surface of the nucleus becomes irregular and its membrane indistinct. Such a condition, no doubt, permits the migration of the nucleoli into the cytoplasm of the cell. Figures 3, 10, and 14 show a number of very large inclusions, which are, no doubt, secretory material. It is very evident that the nucleoli migrate from the nucleus into the cytoplasm (figs. 6 and 9) and increase in size for a time before they are finally dissolved and passed out as a liquid secretion into the lumen of the gland. This explanation would readily account for the streaming movements and the vacuoles which are frequently present in the cytosome. Usually, the nucleoli which are farther away from the nucleus and presumably the oldest are considerably larger than those which are still in the immediate vicinity of the nucleus (fig. 14)—a phenomenon which suggests that both nucleus and cytoplasm are directly responsible for the formation of the secretory substance. Near the outer extremity of the nucleus, some of the nucleoli have enlarged and taken on a more spherical form (figs. 3

and 6). This suggests that the immediate contact with the cytoplasm causes a definite morphological as well as chemical change within the migrating nucleoli, as suggested by Nakahara and others.

Numerous vacuoles are often present in the outer half of the gland (figs. 3 and 9). These have also been noted by Korschelt, who always figures these structures on the inner margin of the gland, while Marshall and Vorhies observed them in the outer area of the gland. Gilson ('90) found vacuoles present in the nucleus and also in the cell body, as did Maziarski. Gilson interpreted them as containing secretory material. Matheson and Ruggles ('07) found that the vacuoles became more abundant during the time of active glandular secretion and the contents remained unstained with any of the coloring agents used. Nakahara has likewise observed vacuoles present in the cytoplasm, and he felt that these were unassociated with the process of secretion as held by Gilson. Our interpretation of the presence of these vacuoles during the periods of secretion is that they are the direct result of the dissolution of the previously described secretory inclusions which have originally migrated in part from the nucleus. The vacuoles in our material are distributed just as the secretory inclusions and are of the same general size. It is interesting to note that surrounding most of the secretory inclusions are clear areas which give the appearance of vacuoles (figs. 6 and 14); however, these might be a direct result of fixation. These figures compare well with the condition pictured by both Maziarski and Ludford.

It is somewhat surprising to note the slight change in the Golgi apparatus. There is no apparent polarity in relation to the nucleus or to the lumen of the gland. The bodies possess the same general appearance and seem to be about the same in number as in glands active for twenty-four hours. As to the direct relationship of the Golgi apparatus to the secretory inclusions, little can be said. There is apparently no direct connection between the Golgi apparatus and the secretory substance, as described by Nasonov, Bowen, Ludford,

and others. One observes but little more direct connection of the Golgi apparatus than he would expect to find as a result of the laws of chance. In figures 6 and 13 the nuclear membrane has become indistinct and the migrating nucleoli have reached the edge of the cytoplasm. A few small black bodies which give the appearance of Golgi material are present on the circumference of some of these nucleoli which would tend to bear out the interpretation of the recent findings of Nassonov ('23) and Bowen ('24) that these small portions of Golgi material serve as centers of synthetic growth of the secretory inclusions. However, this condition occurs as an exception rather than a rule, and therefore cannot be looked upon as convincing evidence in supporting the contentions of these two investigators.

Glands active for longer periods of time

In glands active for a sixty-hour period hardly any inclusions are to be found in the cytosome, but instead, a large number of vacuoles appear in the peripheral area of the gland (fig. 11). Associated with these vacuoles numerous streaming effects are set up in the cytoplasm directed toward the lumen of the gland. Glands active for more than sixty hours differ only slightly from those just described for sixty hours. There is, however, a progressive decrease in the number of nucleoli up to 360 hours of activity. Clear spaces which may or may not be of a vacuolar nature are frequently observed in the nucleus, due, probably, to the emigration of the nucleoli. In some instances the nuclei of glands active for 360 hours show more nucleoli, though smaller in size, than those active for about 200 hours, which would point to the fact that the nucleoli might be formed in cycles. Vorhies ('08) has quite conclusively shown that the new nucleoli are derived from division and growth of preexisting nucleoli of the normal type. He likewise pointed out that the nucleolar material increased with the activity of the cell. On the other hand, Noel and Paillot believe that the nucleoli are derived directly from the chromatin material. Figures 4 and 7 show

large vacuoles which are apparently in immediate association with the nucleus. Careful analysis shows, however, that they are probably outside of the nucleus, but were, in the beginning, nucleoli, which were in the process of migrating into the cell body and have become dissolved *in situ*.

In glands active for 360 hours, which have practically stopped secreting, numerous large vacuoles are present with their long axes directed toward the lumen of the gland (figs. 4 and 7). No secretory inclusions are present, as the supply has been exhausted by the long period of activity. It is quite apparent that the continued activity caused by removal of the case daily for a period of about 360 hours completely exhausts the supply of secretion. Death of the larva usually follows. In such larvae chromatin granules show very little change from those of the normal gland.

There is a slight change noticeable in the Golgi apparatus in glands active for 360 hours (fig. 7). The Golgi bodies have become still smaller and slightly more dispersed than in glands of twenty-four or forty-eight hours of activity. They have no apparent relation to the vacuoles as observed by Nasonov for the contractile vacuoles in protozoa. In no case, from the normal to the gland active for 360 hours, was there observed a true Golgi material in the secretion in the lumen of the gland as described for milk secretion by Beams ('27), although parts of the Golgi material were observed in the secretion just as it was passing from the gland into the lumen. In general, however, there is very little evidence that the Golgi material passes out from the gland with the products of secretion.

DISCUSSION

During recent years, a number of views have developed as to the various processes involved in the mechanics of secretion. From the cytological viewpoint, the cases in which the secretion is derived from the nucleus are fairly limited. The work of several investigators has shown quite conclusively that, in certain cases at least, part of the secretory substance

is derived directly from the nucleus of the cell (Garnier, '00, on the salivary gland of the rat; Maximow, '01, on salivary glands of the dog; and also the work of Schreiner, Maziarski, Nakahara, Kinney, Ludford, Noel and Paillot, and others).

The evidence presented in this paper supports the view that certain of the nucleoli migrate into the cell body and form part of the secretory substance of the cell. Marshall and Vorhies ('06) apparently did not observe this phenomenon, although it was later suggested by Vorhies ('08) that this might be the mode of secretion.

This theory of secretion, however, has not been accepted by some of the most prominent cytologists. Heidenhain ('07) did not think that there was sufficient evidence, from the morphological point of view, to warrant its acceptance. Bowen ('25) expressed the opinion that the problem of secretion, for the cytologist at least, is definitely located in the cytoplasmic area of the cell and agreed with Heidenhain that the nucleus might play only an intermediary rôle in the process of secretion by virtue of its 'regulative potencies.' Wilson ('25) likewise expressed his skepticism as to this mode of secretion and is awaiting further evidence before its acceptance.

Ever since the pioneer work of Altmann, mitochondria have been figured as an important factor in the formation of secretion. This theoretical consideration has been confirmed by Meves, Rugard, Duesberg, Hoven, and others. Mitochondria have been reported in the spinning glands of insects by Kinney ('26), Noel and Paillot ('27), but no definite connection with secretion has been proved.

Probably the most popular interpretation of the secretory phenomenon in gland cells at the present time is that of the definite association of the secretory granules with the Golgi apparatus. The recent findings of Nasonov, Bowen, Ludford, Brambell, and others show conclusively that the Golgi apparatus, in some glands at least, plays an essential part in secretion. The evidence offered by the spinning glands of the caddis-fly larvae on the relationship of the Golgi apparatus

to the secretory process is not sufficient to draw any definite conclusions. There may be some intermediary rôle played by the Golgi apparatus in the process, but certainly no definite connection is apparent as in secretion in the pancreas and glands of the intestinal tract and in the formation of the acrosome of the animal sperm.

It is interesting to note that in the spinning glands of the caddis-fly larvae there is no definite polarity shown by the Golgi apparatus in relation to the large amoeboid nucleus. It was expected that during the active period of the gland the secretory polarity would be fixed as in the acinus of the pancreas and the cells of the salivary glands, where the Golgi apparatus is invariably placed between the nucleus and the lumen of the gland. If the Golgi apparatus is vitally concerned in the formation of secretory products, it would seem not unreasonable to suppose that it might present obvious morphological changes during the different periods of physiological activity.

We have shown that glands of the caddis-fly larvae active for any considerable time show definite vacuoles present in the peripheral areas of the gland. Gilson, and Marshall and Vorhies have likewise noted these structures in both the nucleus and cytoplasm, although disagreeing as to their possible function. Gilson expressed the opinion that the vacuoles were directly connected with the secretory process, while Marshall and Vorhies maintained that they were unassociated with the secretory phenomenon. We cannot altogether agree with these authors that there are definite vacuoles formed in the nucleus. Figures 4 and 7 show, however, that certain vacuoles are very closely connected with the nucleus. We consider that before the nucleoli can become dissolved into secretory material they must be completely, or in part, in the cytoplasm. This location is a prerequisite to the appearance of vacuoles. Our material shows clear areas surrounding the migrating nucleoli which we believe to be of vacuolar nature. This condition agrees well with figures 6 and 7 of Maziarski's paper ('11). It is, we think, a logical

interpretation to assume that the vacuoles which appear in the outer area of the gland are a direct result of the dissolution of secretory inclusions. Our evidence for this interpretation is that the vacuoles vary in size, number, and position just as the secretory inclusions found in actively secreting glands. Moreover, the definite streaming effect leading from regions of the vacuoles toward the lumen of the gland substantiates the interpretation that they were at one time secretory inclusions.

We cannot agree with the recent contentions of Parat ('24) and his collaborators that the Golgi apparatus is a vacuome as described by them in the salivary glands of the *Chironomus* larvae. An attempt was made to impregnate the Golgi apparatus in the spinning glands of *Platyphylax* by Parat's vital method, but no structures were found to resemble in any respect the classical Golgi apparatus as shown by the osmic-tetroxide methods. We are in agreement with Bowen and others that the Golgi apparatus is a definite morphological structure, and not a vacuome as described by numerous French investigators.

The evidence in this paper shows that there are two definite types of bodies present within the nucleus of the spinning glands of *Platyphylax* larva, namely, chromatin bodies and nucleolar bodies. The chromatin bodies, which are smaller than the nucleolar bodies, are usually constant in size, number, and position. The nucleolar bodies, on the other hand, show a wide variation in size, number, and shape. During the pioneer researches of Korschelt ('96) and Meves ('97), a discussion arose as to just which of these bodies constituted the true chromatin material. Korschelt contended that the large particles (macrosomes) were the chromatin material and the small bodies (microsomes), the true nucleoli. Meves ('97) showed the microsomes of Korschelt to be chromatin granules and the macrosomes, the nucleoli. Subsequent researches of Flemming, Henneguy, and Marshall and Vorhies have definitely substantiated the contentions of Meves. More recently, the scene of discussion has been shifted from the

morphology of these bodies to their probable origin within the nucleus. Vorhies ('08) put forth convincing evidence to show that the nucleoli were derived directly from the division of preexisting nucleoli. Noel and Paillot ('27) concluded in their study on the sericigenous glands of *Bombyx mori* that "Le renouvellement des nucléoles ainsi émigrés est assuré par la transformation progressive des mottes chromatiques, incluses dans le noyau, en nucléoles acidophiles." Our observations do not permit us to make any conclusive statement concerning the probable origin of the nucleoli. Some of our preparations show a definite relation between chromatin and nucleoli, while in others absolutely no relationship could be determined. The solution of this point must obviously await further investigation.

It seems unadvisable to offer a general statement concerning a universal phenomenon of secretion with a number of such conflicting views at hand. It would be our suggestion, however, that the process of secretion is not alike in all types of glands, which would readily account for the numerous theories that have developed in connection with this process. This leads us to a conclusion with Ludford that "The cell as a whole is a functioning unit, and we are probably approaching nearest to the truth in studying the changes occurring in its visible structure, rather than seeking to attribute substances formed by it to the activities of any one of its component parts."

CONCLUSIONS

1. A general increase in diameter is accompanied by activity in the spinning glands of *Platyphylax designatus* Walker.
2. In the spinning glands of the larvae of *Platyphylax designatus* Walker the nucleoli migrate into the cell body, enlarge, undergo dissolution, setting up streaming effects in the cytoplasm, and forming at least a part of the secretory products of the cell.
3. In the outer area of the gland appear numerous vacuoles which increase in size and number with increased periods of

activity. These we interpret to be the remains of dissolved secretory inclusions.

4. There exists in the spinning glands of *Platyphylax* a true Golgi apparatus which presents itself in the form of rings, loops, or comma-like structures.

5. The Golgi apparatus is evenly distributed throughout the gland and shows no apparent hypertrophy during the different periods of physiological activity of the gland.

6. The Golgi apparatus shows no apparent relationship to the secretory phenomenon, such as direct connection with the secretory inclusions, definite fixed polarity, or hypertrophy during activity of the gland.

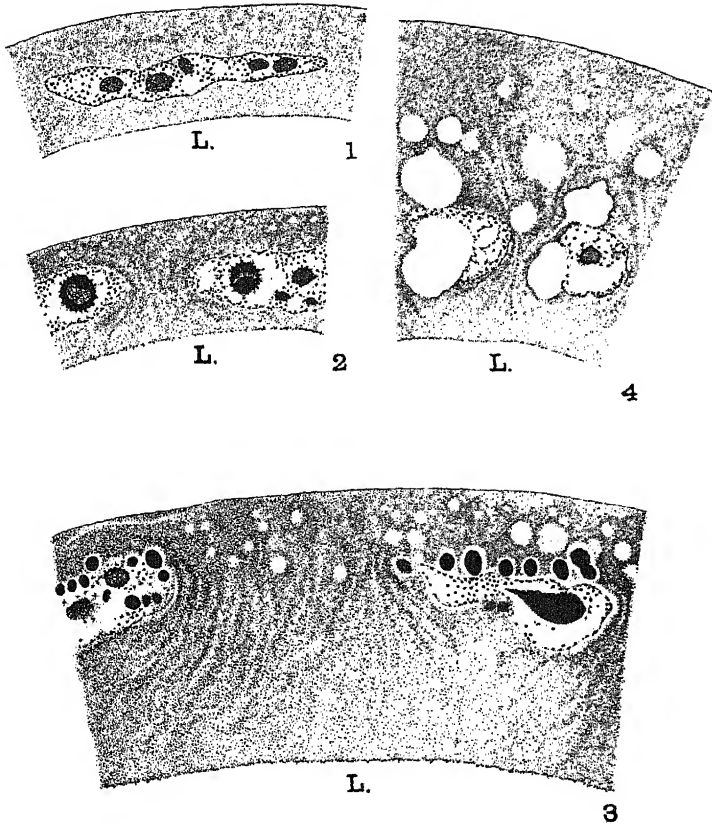
7. Only a small amount of the Golgi material is extruded from the cell body with the products of secretion.

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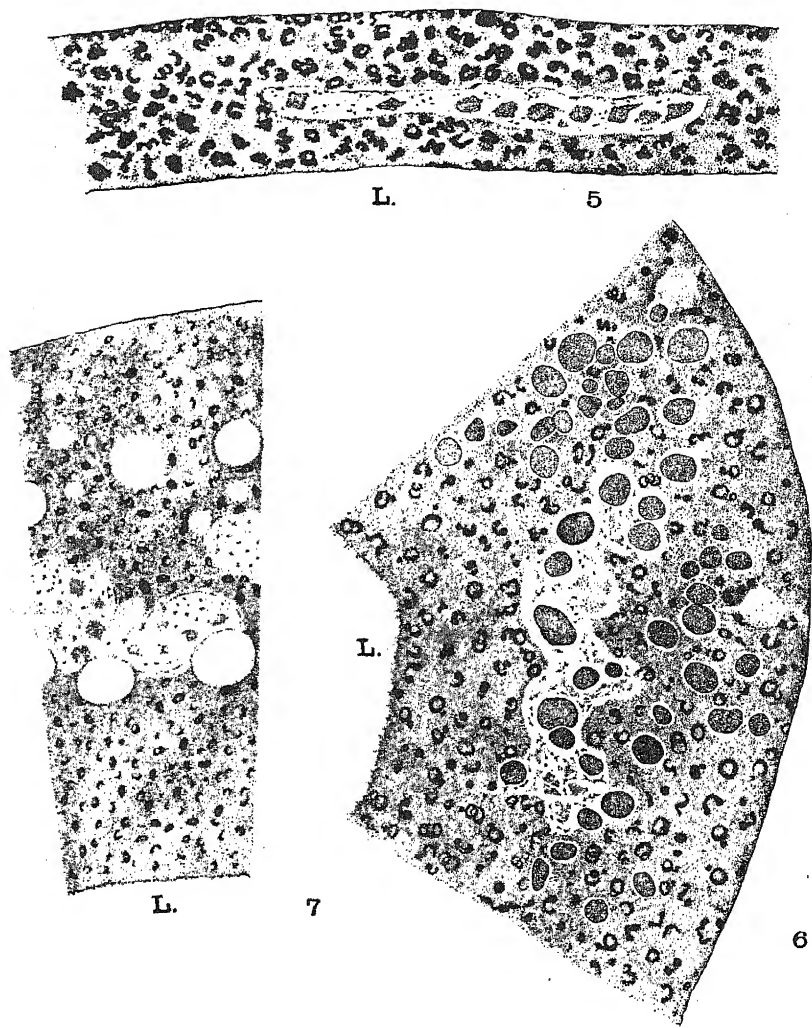
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Figures 1 to 4 were made with the aid of a camera lucida at an initial magnification of approximately $\times 1200$ (reduced one-half in reproduction).

- 1 Normal gland. Cross-section of the secretory portion of a gland in which the case of the larva had not been removed.
- 2 Cross-section of a portion of a gland active for thirty-six hours.
- 3 Similar section of gland active for forty-eight hours.
- 4 A portion of a gland active for 360 hours, after which the larva had completely stopped rebuilding new case.

L, lumen side of gland.

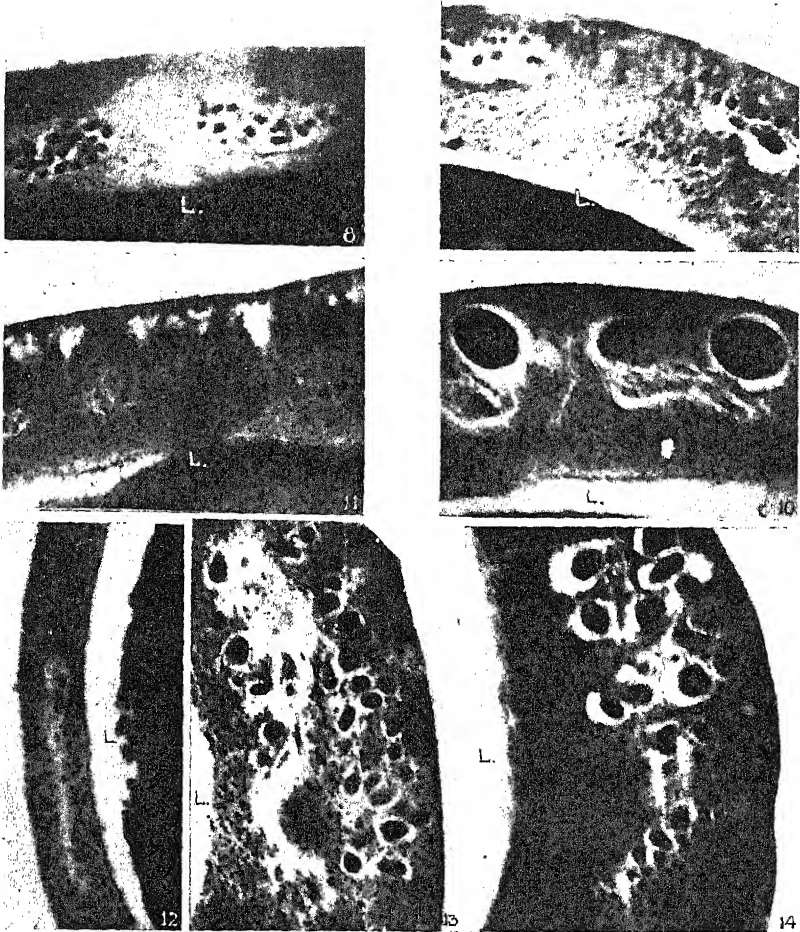


Figures 5 to 7 were made with the aid of a camera lucida at an approximate magnification of $\times 1500$ (reduced one-half in reproduction).

5 Golgi apparatus and nucleus in a normal or resting condition.

6 Cross-section of a portion of gland active for forty-eight hours. Note migration of nucleoli into cell body and distribution of the Golgi bodies.

7 Similar section of gland active for 360 hours. Note vacuoles.



Photomicrographs made at an initial magnification of approximately $\times 500$ (reduced approximately one-fifth in reproduction).

8 Portion of a cross-section of normal gland.

9 and 10 Portions of gland active for forty-eight hours, in cross-section.

11 Portion of a cross-section of gland active for sixty hours. Note vacuoles and streaming effects of cytoplasm.

13 and 14 Similar sections of gland active for forty-eight hours, showing distribution and relation of Golgi apparatus to the secretory inclusions.

THE SIGNIFICANCE OF THE ULTIMOBRANCHIAL BODY (POSTBRANCHIAL BODY, SUPRAPERICARDIAL BODY): A COMPARATIVE STUDY OF ITS OCCURRENCE IN URODELES

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TWO TEXT FIGURES AND FIVE HELIOTYPE PLATES

AUTHOR'S ABSTRACT

The significance of the ultimobranchial body has been the object of a comparative study of the structure in twenty-four species of urodeles. In nineteen of these it has not hitherto been described.

Caudal to the last branchial arch, it develops as a thickening and later as an outpushing from the ventral wall of the pharynx. Due to the growth mechanics of the region, it comes to lie obliquely to the pharynx, ventral to it, and dorsal or dorsolateral to the pericardial cavity in its anterior region. It persists throughout life as an epithelioid or epithelial structure, usually of irregular shape, frequently containing vesicles; in some cases it exhibits a considerable amount of secretory activity of variable quality. Except in *Amphiuma* and *Necturus*, where it is regularly paired, and in occasional instances in individuals of other species, where it occurs on both sides, it is usually present on the left side only. Its occurrence is constant in all of the species of urodeles for which it has been examined.

It is variable in size, form, and position. This, together with the quite inconstant indication of secretory activity, marks it as a structure of little or no physiological significance. 'Colloid' is, however, present in some instances, and hence a comparison with the thyroid was considered.

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INTRODUCTION

The varied development, differentiation, and fate of the ultimobranchial body throughout the vertebrate series make it of peculiar significance and render it subject to different interpretations. It is known to be present among all of the larger subdivisions of each of the classes of vertebrates, and new accounts of it are being given from time to time for various forms, especially among the birds, reptiles, and mammals. Inasmuch as a comprehensive description of this structure in the urodeles is not available, a comparative study has been undertaken with a view to ascertaining the resemblances and differences in its occurrence, as well as its morphologic and general histologic character in a large number of species, an attempt being made in this review to characterize the structure not only specifically, but generally for this subclass.

In summarizing its general occurrence preliminary to a discussion of it in the urodeles, one finds that it has not been described for the cyclostomes. Maurer, in 1902, reviewed the occurrence of the ultimobranchial body, noting its presence among the fishes, except in *Heptanchus* of the elasmobranchs and among the teleosts. Although these exceptions were again mentioned by Baldwin in 1918, Braus ('06) had meanwhile described the ultimobranchial body in *Heptanchus*. As Camp ('17) points out in his article on the development of the suprapericardial (ultimobranchial) body in *Squalus acanthias*, Nussbaum-Hilarowicz ('16) has described in *Stomias boa* a small paired gland, ventral to the pharynx, which corresponds very closely to the ultimobranchial body of the other teleosts among which it has been found and can be homologized with that of selachians. In Amphibia, Greil ('05) noted its absence in *Bombinator*—an exception to the general rule found by Maurer among the Anura. In this investigation it was found without exception in urodeles. Although it was reported by van Bemmelen ('86) to be entirely absent in some cases among the snakes, it otherwise occurs without known exception among the reptiles, birds, and mammals. Recent work among the reptiles includes a description of the ultimo-

branchial body in the turtles by Johnson ('20, '22) and Shaner ('21); among the birds: in the duck by Rabl ('07), in the sparrow by Helgesson ('13), in the grebe by Johnson ('18), and in the lapwing by Sicher ('21); among the mammals: in the human by Grosser ('10, '12) and by Kingsbury ('14), in the rabbit by Kohn ('95), in the guinea-pig by Rabl ('22), in the pig by Badertscher ('18, '19), in the cat by Stewart ('18), and in the rat by Rogers ('27).

In all cases the ultimobranchial body occurs in the embryo as the most caudal outgrowth of the epithelium of the pharynx behind the last branchial arch. Although universally constant in this relationship, it varies in its asymmetrical or paired appearance. Behind the sixth arch in elasmobranchs it is sometimes paired and sometimes single. Behind the fifth arch in Amphibia it is usually paired in Anura, while in the urodeles it is found to be in most instances single, appearing on the left side, but occasionally paired, and constantly so in *Amphiuma* means and *Necturus maculosus*. Occurring behind the fourth or fifth pouch in the reptiles, it is sometimes single, sometimes paired; in the birds, as in some of the reptiles, it develops as a paired structure, but in some cases, as in the sparrow, the body on the right side atrophies; in mammals it appears uniformly on both sides behind the fourth pouch, except in the rat, where the third pouch is the last (Rogers, '27).

If, then, this structure is truly ultimobranchial, it cannot be directly homologized throughout the different classes of vertebrates, where the number of arches and pouches in the series may vary. This was Kingsbury's ('14) objection to Rabl's ('11) suggestion that in mammals it represents a fifth and sixth pouch (sixth in guinea-pig) and to Verdun's view, later expressed by Grosser, that it is a ductless gland which has become rudimentary.

Maurer ('11), who introduced the term 'postbranchial' for the ultimobranchial body, and Verdun ('98), in considering it a 'postbranchial' structure, found no difficulty in homologizing the body in the different classes. Kingsbury ('14),

however, considers it of branchial, and not 'postbranchial,' origin, representing no specific pouch, but merely formed by "a continued growth activity in the branchial entoderm." Emphasis on its relationship to the pericardium rather than to the pharynx and branchial structures caused van Bemmelen in the selachians and Platt in *Necturus* to term it 'suprapericardial.' It had been confused with the thyroid and accordingly called the 'accessory thyroid' by de Meuron ('86). That the structure of the ultimobranchial body sometimes resembles that of the thyroid is evident in the case of *Triton cristatus*, which is later described. The term 'postbranchial,' introduced by Maurer, has been accepted by others, including Lillie, Baldwin, Shaner, and Uhlenhuth. The term 'ultimobranchial,' introduced by Greil ('05), who found that the structure in anurous *Amphibia* develops from a last branchial pouch, has been adopted by Marcus, Rabl, Kingsbury, Johnson, Stewart, Badertscher, Sicher, and Rogers.

While in the urodeles the ultimobranchial body is found in some details to resemble the thyroid, in the case of certain mammals it forms an intrinsic part of the gland itself, as Badertscher ('18, '19) finds in the pig, where the ultimobranchial bodies contribute to thyroid structure instead of undergoing degeneration. Rogers ('27) finds in the albino rat that, after becoming embedded in the lateral lobes of the thyroid, the ultimobranchial body fuses with it and is transformed into thyroid or thyroid-like cords which are not distinguishable morphologically from those arising from the median thyroid anlage. Later, colloid-containing follicles appear in the region occupied by these ultimobranchial cords. They constitute, however, only a small part of the thyroid gland, as Badertscher has also stated for the pig.

Instead of contributing to thyroid structure, in the cat the ultimobranchial body undergoes thymic transformation, according to Stewart ('18, p. 214). Lillie ('19) also states that in the chick the postbranchial bodies form "two main kinds of epithelial tissues similar to the tissues of the thymus and epithelial vestiges respectively."

In the urodeles, however, the ultimobranchial body does not undergo thymic transformation and is quite apart from the thyroid in position, although there are superficial structural resemblances to the latter, as is shown subsequently.

'Secretion' within the follicles of the ultimobranchial body is found in other vertebrate classes. Among the elasmobranchs Camp ('17) finds that in *Squalus acanthias* the expanded ventral portion of the gland consists of "large, distended vesicles, most of which intercommunicate, but some of which are completely isolated." He describes these vesicles as having a lining consisting of a single layer of narrow columnar cells which actively secrete mucus. Maurer ('88) finds the bodies in *Anura* containing either a single large follicle or a complex of smaller ones, which during some developmental stage contain a serous (watery) secretion, but never colloid. In the turtles, *Chrysemys marginata* and *Chrysemys picta*, Shaner emphasizes the fact that a colloid-like secretion is present in the postbranchial body—contrary to Maurer's ('99, '02) statement that colloid is absent in all non-mammalian vertebrates, appearing first in *Echidna*.

According to Johnson, in the pied-billed grebe among the birds, the left postbranchial body, which alone of the two persists in an embryo of twelve days, consists of a relatively large irregular mass lying upon the dorsal side of the thyroid. Epithelial vesicles have not been formed at the time. Verdun ('98), summarizing the facts known about the ultimobranchial body of birds in general, finds that it is composed of cords and epithelial lobules, separated by a moderately abundant amount of connective tissue that is quite vascular. It contains spherical or irregular cavities lined by cuboidal or columnar epithelium, ciliated in places in the duck. In addition, a small gland and thymic tissue are found with the parenchyma or completely surrounded by it.

In spite of the presence among certain of the urodeles of secretion within vesicles which in some cases closely resembles thyroid colloid, the ultimobranchial body is probably, as Uhlenhuth considered in *Amblystoma opacum*, of little physio-

logical importance. Except for Uhlenhuth's observations, the work among the urodeles has been chiefly directed toward a study of the morphology and general histologic character of the ultimobranchial body.

In those urodeles where development has been studied, the ultimobranchial body exists first as a mere thickening of the epithelium of the pharynx behind the last branchial arch. It develops as a ventral outpushing which soon separates from the parent tissue, protruding vertically downward toward the dorsal wall of the pericardium, and, enveloped in connective tissue, sinks to a deeper position, coming to lie above and dorsal to the anterior end of the pericardium. In general, among the urodeles it possesses a vesiculated, branched structure, which shows throughout its extent the presence of cavities, seldom very large, which are often not continuous with each other. Little cytoplasm is present in its cells.

Recent work on the ultimobranchial body in the urodeles has been carried out by Baldwin and by Uhlenhuth and McGowan. The development of the 'postbranchial' body in *Amblystoma punctatum* is described in detail by Baldwin ('18, p. 639).

Although Baldwin does not find the ultimobranchial body in old heads of *Amblystoma punctatum*, Uhlenhuth and McGowan ('24), working on *Amblystoma opacum*, find that the postbranchial body persists throughout life, up to an age of four and one-half years. They find (p. 601) that it increases only slightly in absolute weight throughout life, but shows a marked decrease during metamorphosis.

With these various interpretations of accumulated data in mind, a study of the ultimobranchial body among the urodeles was undertaken. In certain of these forms its development has been traced; in others notes on its occurrence and structure in typical stages have been made. In still other forms only one or two representatives of the species have been accessible for study. A consideration of its occurrence has shown it to be universally present throughout the group studied, there being no case in which it is not found, although a group

of epithelioid cells may be all that marks its presence. Its relationship to surrounding structures, found to be quite generally similar in all of the species examined, was noted, while its variations in size and symmetry, and its capacity (or lack of it) to form secretion have all been kept under consideration.

OBSERVATIONS¹

General

From a survey of the work previously done in the group of urodeles it is evident that the ultimobranchial body has not been described for a sufficient number of forms to characterize it generally for this subclass. So far as it has been cited in literature, it is known to occur in *Salamandra maculata*, in *Typhlomolge rathbuni*, in three species of *Amblystoma*, and in three species of *Triton*. Moreover, its constancy for a larger number of individuals in these species is for the most part not known. Whether it is of regular occurrence or whether it is sometimes absent cannot be predicted for all forms from the data already recorded. Baldwin ('18) claims that in old adults of *Amblystoma punctatum* there is sometimes no evidence of it. Concerning its glandular nature little is known. Maurer is the only one who has found secretion within its vesicles, yet he emphasizes its dissimilarity to thyroid colloid. The wide range of material available for this study affords an opportunity to consider these points.

A large number of forms and variety of stages among them included chiefly series of transections, with a few frontal and

¹I wish to express my gratitude to Prof. B. F. Kingsbury, at whose suggestion this work was undertaken, for the encouragement and advice which he has given me throughout the progress of this investigation.

The extensive material utilized in this study was drawn from several sources, especially from the collections of Prof. Simon H. Gage, the Laboratory of Histology and Embryology of Cornell University, and the Laboratory of Zoölogy of Cornell University. Hence, I desire to express to Prof. S. H. Gage, Prof. B. F. Kingsbury, and Dr. H. B. Adelmann, of the Department of Zoölogy of Cornell University, Dr. R. R. Humphrey, of the University of Buffalo Medical School, and Dr. L. Hoadley, of the Department of Zoölogy of Harvard University, my appreciation of their generous loans.

sagittal series. In their examination a more definite concept of the morphologic and physiologic nature as well as the development of the ultimobranchial body could be formed. Series covering the range of developmental and adult stages were available in the case of *Necturus maculosus*, *Amphiuma means*, *Cryptobranchus alleghehiensis*, *Amblystoma punctatum*, *Triturus viridescens*, *Salamandra atra*, *Desmognathus fuscus*, *Plethodon cinereus*, *Hemidactylium scutatum*, and *Eurycea bislineata*. In some of the other species, however, there were only a few series examined, and here the structure of the adult ultimobranchial body was chiefly all that could be determined. In certain species, such as *Rhyacotriton olympicus* and *Typhlotriton spelaeus*, but a single specimen in each case was examined.

As has been already intimated, the ultimobranchial body has hitherto been reported for relatively few urodeles. The accompanying table (fig. 1) presents in a general way the information gained in this investigation for such species of tailed Amphibia in which this pharyngeal derivative has not been previously described. It may be noted that in the large salamander *Amphiuma* (as in *Necturus*) both right and left ultimobranchial bodies are present. The table further suggests that, were enough individuals examined, the occasional occurrence of a right ultimobranchial body in any species would be found. Detailed comments on the relations in these species follow.

The characteristics of the ultimobranchial body in urodeles

Location. In the urodeles the ultimobranchial body, usually occurring on the left side only, is caudal to the last gill arch, between it and the aditus laryngis, ventral to the pharynx, and dorsal or dorsolateral to the pericardial cavity in its anterior region. It is in the proximity of the *m. transversus ventralis*, which underlies the pharynx, the *m. thoracicohyoideus*, which is lateral to the pericardial cavity, and the last aortic arch to which in many species it is adjacent. This position, characteristic of the ultimobranchial body in the urodeles, is shown

in a 43-mm. *Plethodon cinereus* (fig. 3). There is some variation in its cephalocaudal position; occasionally it is anterior to the pericardial cavity, extending caudally from a plane

FORM	NUMBER			PRESENCE	
	Embryo and larva	Transforming	Adult	Right	Left
Amphiumidae:					
<i>Amphiuma means</i>	5	1		6	6
Cryptobranchidae:					
<i>Cryptobranchus allegheniensis</i>	19	2	1	0	22
Salamandridae:					
<i>Triturus torosus</i>	2	1	1	0	4
<i>Triturus viridescens</i>	1	3	1	0	5
<i>Salamandra atra</i>	6	13	8	1	27
Amblystomidae:					
<i>Rhyacotriton olympicus</i>			1	0	1
Plethodontidae:					
<i>Batrachoseps attenuatus</i>	3		1	0	4
<i>Hemidaetylium scutatum</i>	30		1	0	31
<i>Plethodon cinereus</i>	21	8	33	1	62
<i>Plethodon glutinosus</i>			1	0	1
<i>Stereochilus marginatus</i>	1		1	0	2
<i>Gyrinophilus porphyriticus</i>		2	1	0	3
<i>Pseudotriton ruber</i>		3	1	0	4
<i>Eurycea bislineata</i>	14	10	7	0	31
<i>Desmognathus fuscus</i>	33	5	2	2	40
<i>Desmognathus ochrophaeus</i>	3			0	3
<i>Typhlotriton spelaeus</i>			1	0	1
Sirenidae:					
<i>Siren lacertina</i>			2	0	2
<i>Pseudobranchius striatus</i>			2	1	2

Fig. 1 Table showing the occurrence of the ultimobranchial body in the larval, transforming, or adult stages in the species not hitherto examined. The occurrence on right or left side is given.

passing through the branching of the truncus arteriosus, rarely being anterior to this. Although in all of the species of urodeles studied the ultimobranchial body is similarly derived from the endoderm of the floor of the pharynx, its dis-

tance from it varies, not only according to the developmental stage of the individual, but also according to the structural development of the *m. transversus ventralis* and the *m. thoracicohyoideus* as well as other factors involved in the growth mechanics of the region. Because of the variation in shape and extent of these muscles among different species of urodeles, the ultimobranchial body may be found sometimes occupying a lateral position near the cartilage of the last branchial arch, sometimes nearer the median plane and the aditus laryngis. In *Pseudobranchius striatus* (of which two adults were examined) it is extremely lateral in position; in *Plethodon cinereus* it lies nearer the median plane.

In a late embryonic stage of *Cryptobranchus allegheniensis*, as in most of the species examined, the ultimobranchial body lies midway between the lateral border of the *m. transversus ventralis* and the cartilage of the last arch, extending nearly to the pericardium. In the majority of embryos and early larval stages examined in this species, a similar position is retained, except for the assumption of a more dorsal position after connection with the pharynx has been lost. In a 34-mm. larva it lies 100 μ from the pharynx and away from the pericardium; in later stages it remains just beneath the floor of the pharynx, being kept in this position by the dorsal expansion of the *m. thoracicohyoideus*. This is evident in a 45-mm. *Cryptobranchius* (fig. 23), where it is also neither in a medial position beside the *m. transversus ventralis* nor laterally in contact with the cartilage of the last branchial arch, but lies halfway between the two. In the adult the body has been forced by the dorsal growth of the *m. thoracicohyoideus* laterad and closer to the cartilage of the arch.

In *Eurycea bislineata* (fig. 26), likewise, the *m. thoracicohyoideus* is very large and presses the ultimobranchial body against the floor of the pharynx, although the thin lateral portion of the *m. transversus ventralis* is between the two. In younger individuals, as in a 15-mm. larva, it is at the side of, rather than ventral to, the latter muscle and extends dorso-laterad from the pericardium with which it is in contact, com-

ing to lie nearly against the pharyngeal epithelium, yet dorsal to the *m. thoracicohyoideus*. In a 25-mm. *Eurycea bislineata* the ultimobranchial body becomes more median as the *m. thoracicohyoideus* grows dorsally. In a 31-mm. individual of the same species it is found in contact with the pericardium, due to the expansion of this muscle and the *m. transversus ventralis*. Lying between the two structures just mentioned in a 55-mm. larva of *Pseudotriton ruber*, the body is, however, lateral to the *m. transversus ventralis* and nearer the cartilage of the last branchial arch.

The ultimobranchial body is commonly found lateral to the *m. transversus ventralis*. In a 30-mm. embryo of *Amphiuma* means (fig. 13), where it is still connected with the pharynx, this is evident; it curves closely around the lateral border of the muscle in an older embryo, although in the adult the position becomes altered (fig. 18) as it comes to lie ventral to the muscle. In a *Necturus* embryo of 20 mm. on either side (in this species a paired structure) it is lateral to the *m. transversus ventralis*, between the pericardium and the cartilage of the last branchial arch. In a 21-mm. *Necturus* embryo, through the lateral expansion of this muscle it comes to lie so that at its posterior end it is in contact with both structures, muscle and cartilage. This is typical of the later position (fig. 5). In this 70-mm. individual, both on the right and on the left, the further growth laterad of the *m. transversus ventralis*, shown in a posterior section, has divided the ultimobranchial body into two portions, one dorsal to the muscle where it comes in contact with the cartilage, the other ventral to it in the same relative position.

Although in young *Triturus* larvae the ultimobranchial body is not in contact with the cartilage of the arch, in transforming larvae it is closely pressed between the two structures, while in a 23-mm. *Triton* larva it seems as a solid stalk to penetrate between the arch and the muscle. It is still at the lateral border of the muscle in the adult. In embryos of *Plethodon cinereus* just ready to hatch and in 12-mm. and 13-mm. larvae the ultimobranchial body lies lateral to the muscle also, although the position in the adult is altered.

In many instances, however, the position of the ultimobranchial body does not seem dependent upon the lateral growth of the *m. transversus ventralis*, for, whether the muscle is in contact with the arch or not, the ultimobranchial body is ventrolateral or ventral to the muscle. It is then removed from the vicinity of the pharyngeal wall, in contrast to its position in an adult *Typhlomolge rathbuni*, where it contains a relatively large cavity and is situated just beneath the pharyngeal epithelium. In a 33-mm. embryo of *Amphiuma* means (fig. 18), where the structure is paired, it is ventrolateral to the muscle on the right, ventral to it on the left. This position relative to the *m. transversus ventralis* may vary throughout the extent of the structure, as in an adult *Stereochilus marginatus* (fig. 17), where anteriorly it is lateral to the *m. transversus ventralis* and posteriorly is ventrolateral to it. Within the species it may also vary, as in a 9.8 mm. (twenty days) *Hemidactylum scutatum* embryo, where its position is that later characteristic of the adult—lateral or ventrolateral to the muscle.

The ultimobranchial body is found in many species in the area between the *m. transversus ventralis*, the *m. thoracico-hyoideus*, and the pericardium. In a 19-mm. *Plethodon cinereus* larva it is ventral to, and in close contact with, the *m. transversus ventralis*; in a 43-mm. individual (fig. 3) it lies, as is typical of it in the adult, between the two muscles and against the pericardium. Other instances where it is found between the *m. transversus ventralis* and the pericardium occur in a 37-mm. *Eurycea bislineata* larva and in a 12.5-mm. larva and a 70-mm. adult of *Salamandra atra*. In the last instances the posterior end is dorsally in contact with the *m. transversus ventralis* and ventrally against the pericardium. In young *Desmognathus fuscus* larvae (14 to 20.5 mm. length) the ultimobranchial body is restricted chiefly to the roughly triangular area between the pericardium medially, the *m. transversus ventralis* dorsally, and the *m. thoracico-hyoideus* ventrolaterally. This is seen, too, in a 30-mm. adult. It is found in the same location in an adult *Typhlomolge*

rathbuni and in *Hemidactylium scutatum* larvae of about 15 mm.

In some instances the ultimobranchial body is not in contact with the pericardium, but is compressed between the m. transversus ventralis and m. thoracicohyoideus, as in a larval *Gyrinophilus porphyriticus* of 82 mm. (fig. 12).

In *Rhyacotriton olympicus* (fig. 22) it follows for 500 μ along the dorsal wall of the last aortic arch between the two muscles, as it does in two adult individuals of *Siren lacertina* (fig. 11). In an adult *Pseudobranchius striatus* (fig. 24) the ultimobranchial body is just ventral to and near the lateral limit of the very wide pharynx, between the extraordinarily large m. thoracicohyoideus and the dorsoventrally flattened m. transversus ventralis, and in contact with the last aortic arch. The ultimobranchial body is found flattened between the muscles, on both sides in a young adult *Amphiuma* means of 53 mm., on the left side only in a 42-mm. larva, as well as in an adult, *Triturus torosus*. In contact with the wall of the blood vessel mentioned, it is also found in *Eurycea bislineata*, as well as in a 12.2-mm. (twenty-five days) larva of *Hemidactylium scutatum*. In transforming larvae of *Salamandra atra* the stalk which stretches from the pericardium to the pharynx usually is found extending along the dorsal wall of the artery, and this is true in the case of a 70-mm. adult, where even in the case of the large follicle present the body is closely applied to the blood vessel. Although the ultimobranchial body is usually found against the dorsal surface of the artery, it comes nearly to surround the blood vessel, except on its posterior surface, while it is in contact on the posterior surface only in *Desmognathus fuscus* larvae of about 26 and 28 mm.

From the preceding description it has been shown that the ultimobranchial body is usually lateral or ventrolateral to the m. transversus ventralis. In a few instances it is dorsal or partly dorsal and partly ventral to the muscle. In two cases where it is paired, in an 86-mm. *Salamandra atra* (fig. 4) and a 33-mm. embryo *Amphiuma* means (fig. 18), on one side it is

dorsal, on one side ventral, to the muscle. Groups of cells constituting a very irregular ultimobranchial body are found also entirely dorsal to the *m. transversus ventralis* in a 21-mm. embryo of *Necturus maculosus*. In a 16-mm. larva of *Batrachoseps attenuatus*, although the ultimobranchial body is anterior to the muscle at its cephalic end, at its level it is dorsal to it, as it is also in a 43-mm. *Plethodon cinereus* at its caudal end, after being lateral to it in its anterior part. In other cases it is never entirely dorsal to the muscle, but is partly dorsal, partly ventral to it, as in the case of a 26-mm. *Plethodon cinereus*, a 28-mm. *Eurycea bislineata*, and some *Desmognathus fuscus* larvae of 15, 18.5, and 19 mm. length. In other *Desmognathus fuscus* larvae of the same size it is found lateral to the *m. transversus ventralis*; in still others it is ventral to it and beside the pericardium. Such variations in position relative to other structures in this region are found not only among individuals of the same species, but also within the individual. In the case of a 33-mm. *Amphiuma* means it is dorsal to the muscle at its cephalic end, lateral to it in the middle of its structure, and ventral to it at its caudal extremity. On the left side in cephalocaudal direction in the 86-mm. *Salamandra atra* previously mentioned, it is in turn ventral, lateral, then dorsal to the muscle.

In the urodeles the position of the ultimobranchial body in relation to the pericardial cavity is characteristically variable. In *Pseudobranchius striatus* (fig. 24) and *Siren lacertina* (fig. 11) it is relatively distant from the pericardium. In 9-mm. (sixteen days) and 9.9-mm. (18.5 days) embryos of *Hemidactylium scutatum* it remains throughout its entire length in contact with the pericardium. In *Triturus viridescens* it is in contact with the pericardium before its connection with the epithelium is lost. In many larvae of *Desmognathus fuscus* 18.5 and 19 mm. in length it is in contact with the pericardium. Between 26 and 28 mm. it is still near the pericardium, but a part has grown out between the *m. transversus ventralis* and the *m. thoracohyoideus*. In a 10.5-mm. *Plethodon cinereus* the ultimobranchial body lies dorsal to the pericardium on a

level with the anterior part of the pericardial cavity, but this position is relatively more posterior than is found in the adult. A 43-mm. stage (fig. 3) is the youngest of this species to show the characteristic position of the structure in contact with the pericardium, conforming to its dorsolateral curvature. In most cases it is the caudal end only which lies beside the pericardium, although a few in the group of adults examined showed it for its entire length closely applied to the pericardium. In many instances it is found running obliquely to the pharynx, with one end near or in contact with the pericardium, the other in the vicinity of the pharyngeal epithelium. In a 33-mm. embryo *Amphiuma means* (fig. 18) its cephalic end touches the pericardium; in several larvae of *Eurycea bislineata*, where it is quite constantly near the pericardium, the caudal end adjoins it. In *Salamandra atra* the greater bulk of the body is often found near the pericardium, with the caudal end sometimes beside it. A similar case occurs in a 23-mm. *Triton cristatus* larva, where the ultimobranchial body is connected with the pharynx for about 45 μ , after which it runs diagonally and caudally in the triangular region between the pharynx, the cartilage of the last branchial arch, and the m. transversus ventralis. It then enlarges dorso-ventrally, extending toward the pericardium. In early transforming stages of *Amblystoma punctatum*, Baldwin states that "as in the previous stages, its anterior end is close to the wall of the pericardium, its caudal end close to the floor of the pharynx."

In the series of urodeles examined the ultimobranchial body remains on a level with the m. transversus ventralis and the anterior region of the pericardial cavity (fig. 3) throughout its entire length. In *Plethodon cinereus* (fig. 19) and *Batrachoseps attenuatus*, however, the cephalic end of the structure is quite constantly anterior to the muscle and the pericardial cavity. Occasionally in *Hemidactylium scutatum* this is also true, as in one adult *Typhlotriton spelaeus* (fig. 27). In an adult *Stereochilus marginatus* (fig. 17) the cephalic end is 180 μ anterior to the m. transversus ventralis. In other

species, as in *Triton cristatus*, it is found on a level with the glottis (figs. 13 and 18) and is often found in sections which pass through the branching of the truncus arteriosus, as in certain individuals of *Eurycea bislineata* between 20 and 31 mm. In a 44-mm. *Salamandra atra* (transforming larva) the ultimobranchial body is found in this same plane extending dorsolaterad along the dorsal wall of the left branch, as it frequently does in *Plethodon cinereus*, having the same relation to this blood vessel in a 12.5-mm. embryo and a 16-mm. larva of *Batrachoseps attenuatus*.

In some of the cases where the ultimobranchial body is paired, the right and left structures are at different levels. Although in a 20-mm. embryo *Necturus maculosus* and in an 86-mm. adult *Salamandra atra* both structures are on the same level, in one of the 33-mm. *Amphiuma* means examined the left body is anterior to the right, and in a later embryo the caudal end of the right body is on a level with the cephalic end of the left structure. Again, in an adult *Plethodon cinereus* the right structure is anterior and not so long as the left, the caudal 50 μ of the right body being on a level with the cephalic 50 μ of the left.

Form. The ultimobranchial body in the urodeles in its simplest structure consists of a group of epithelioid cells. It may consist only of a sheet or strand of cells or even merely a cluster of them. Characteristically, however, it has many vesicles developed within it and often, when these are lacking, appears to have its cells grouped about a potential lumen. In the majority of those species studied the adult structure exhibits to a greater or lesser extent a branching, twisted nature, sometimes being extremely irregular, at other times compact, this regularity or irregularity apparently depending upon the growth and development of the muscles and blood vessels in this region. Its long axis lies usually in a cephalocaudal direction. It tapers at either extremity. More often it is broader dorsoventrally than laterally, although, because of its position between the m. transversus ventralis and the m. thoracico-hyoideus, it may be laterally compressed between the cartilage

of the last branchial arch and one or both of the muscles. That the development of vesicles within the ultimobranchial body does not always appear coordinately with its branching to mark the characteristic adult structure is apparent in those instances where one large vesicle represents the entire body, while in others solid branches develop irregularly with no cavities within them.

In a few instances the ultimobranchial body is a compact structure with regular contour. This is true of it in its early development as a mere thickening. As it grows it remains a short and broad structure in *Hemidactylum scutatum*, as it is also in *Amblystoma punctatum* and *Plethodon cinereus*, although long and finger-like in *Salamandra atra*. As growth continues here it is bent medially, as in 9.4- and 9.9-mm. larvae and some *Amblystoma maculatum* larvae of about 11 mm. It still remains compact, however, as in a 16.5-mm. larva of *Hemidactylum scutatum* (fig. 15), where it is found against the dorsal wall of the pericardial cavity. In *Batrachoseps attenuatus* it is a two-layered strand of cells in a 12.5-mm. larva; in a 16-mm. individual it is still a solidly built structure. In other species it remains compact in older individuals. In *Rhyacotriton olympicus* (fig. 22) it is quite regular in contour, as it lies compressed between the muscles and along the last aortic arch. In *Cryptobranchus allegheniensis* (fig. 23) the ultimobranchial body is compact, consisting of but one vesicle. Somewhat similar is the condition in *Pseudobranchius striatus*, where one vesicle makes up the greater part of the structure. In this species, too, as well as in some *Desmognathus fuscus* larvae of about 17 mm. and in certain other species, a poorly defined ultimobranchial body composed of a compact cluster of cells occurs on the right side as well.

In early larval stages, usually after connection with the pharynx has been lost, the ultimobranchial body becomes branched, although in *Eurycea bislineata* there is little branching in the structure in 15- to 31-mm. larvae. In a young adult *Amphiuma* means of 53 mm., where it lies com-

pressed in the connective tissue between the two muscles, there are no lateral or medial branches of the ultimobranchial body given off. Branching characterizes the adult structure in the majority of the species. In a 45-mm. *Salamandra atra* undergoing transformation there is a very much branched ultimobranchial body, although a broad connection with the pharynx has still been retained. A similar connection with the pharyngeal epithelium is found in an 85-mm. adult *Batrachoseps attenuatus* (fig. 21). In an adult *Rhyacotriton olympicus* (fig. 22) it is chiefly a solid structure containing small vesicles. In *Cryptobranchus alleghehiensis* (fig. 23) it is slightly branched with prolongations of the body extending posteriorly, while in an adult *Triton cristatus* (fig. 7), an adult *Typhlotriton spelaeus* (fig. 27), an adult *Typhlomolge rathbuni*, and a 95-mm. *Gyrinophilus porphyriticus* larva it is a very irregular, loosely knit structure.

The processes or branches of the ultimobranchial body are often parallel to the longitudinal axis of the body in a cephalo-caudal direction, where they form a relatively closely knit structure as in *Plethodon cinereus* (fig. 3). In some instances branches are restricted either to the cephalic or caudal end. Commonly, it is more branched at the latter end, as in *Cryptobranchus alleghehiensis*, where a blood vessel winds between its posterior prolongations, and in a 32-mm. larva of *Salamandra atra* (fig. 20), where the branching is found chiefly at the posterior end. Frequently, the branches extend out from the main body of the structure, as in the case of *Stereochilus marginatus*, where one is found at right angles to the main axis of the body (fig. 17). Not uncommonly, portions of the ultimobranchial body may be found separated from the main structure. In *Desmognathus fuscus* the structure in a 15.5-mm. larva and in others between 26 and 28 mm. shows one isolated portion dorsal to the m. transversus ventralis, between it and the pharynx. In an adult *Siren lacertina* (fig. 11) there are three separate divisions of the structure. These lie in the connective tissue compressed between the m. transversus ventralis and the m. thoracicohyoideus, the last aortic

arch occupying a similar position between the muscles. All of these divisions in an adult *Siren lacertina* show little, if any, vesiculation. This is also true in certain adults of *Amblystoma punctatum* where the ultimobranchial body is not especially well developed. The development of but one vesicle of good size is found in *Cryptobranchus allegheniensis*, *Pseudobranchus striatus*, and in a 140-mm. adult *Gyrinocheilus porphyriticus* where it is at the end of the structure. An 82-mm. larva of the last species (fig. 12) shows only small vesicles throughout its length of 320 μ . Contrary to this, extensive vesiculation characterizes the structure in the adults of *Plethodon cinereus*, apparently without exception, although the vesicles never reach extremely large size nor is their location restricted to any region of the body. The structure of the wall of one of the larger vesicles found in the ultimobranchial body of this species may be seen in figure 9. It is within such vesicles that 'secretion' is found.

The development of vesicles varies not only, as in *Cryptobranchus allegheniensis* and *Pseudobranchus striatus*, according to species, but even among individuals within the species. An adult *Typhlomolge rathbuni* shows the ultimobranchial body consisting of one large irregular vesicle just beneath the epithelium of the pharynx. Throughout its length the structure in *Stereochilus marginatus* contains a slight cavity, whereas in *Typhlotriton spelaeus* only small irregular cavities are seen. One adult *Siren lacertina* shows extensive vesiculation, there being one vesicle much larger than the others. In *Salamandra atra* the vesicles within the ultimobranchial body attain only a medium size, yet in the three instances mentioned (one transforming larva, a 70-mm. and an 83-mm. adult) the cavity becomes greatly enlarged, in each instance being filled with secretion. That in the transforming larva is shown in figure 14, while figure 6 shows such a vesicle under higher magnification as it appears in one of the adults, being of a similar structure in the other adult.

Although a cavity continuous with that of the pharynx characterizes the developing ultimobranchial body of *Crypto-*

branchus allegheniensis, in other species lumina within the body do not usually develop until a separation from the pharynx has taken place. In *Desmognathus fuscus* vesiculation characteristic of the adult appears in a 27-mm. individual. At 30 mm. the cavities are large-sized. In *Plethodon cinereus* a very small lumen appears within the ultimobranchial body of an embryo just ready to hatch. Larger, irregular vesicles are found in a 14-mm. individual and in them slight traces of secretion can be detected. By the time a length of 19 or 20 mm. is attained, the structure consists of hollow processes, and these are characteristic of the adult (fig. 10). In *Hemidaetylum scutatum* the first evidence of a lumen occurs at 11.1 mm. In a 21-mm. *Salamandra atra* the ultimobranchial body is very vesicular and it appears to be of a similar structure in older individuals of this species—individuals in which vesicles of extremely large size are developed having been previously mentioned. Apparently, from the one adult examined the structure in *Triton cristatus* is similarly vesiculated.

Although no lymph spaces are found associated with the ultimobranchial body in the urodeles, contrary to Baldwin's observations in *Amblystoma maculatum*, there is in many species a characteristic blood-vascular supply. Often the entire structure from anterior to posterior extremity is associated with a capillary plexus which extends around and between the branches of the body, as is shown in a 32-mm. *Salamandra atra* (fig. 20) and as also appears in a transforming larva of the same species, an adult *Rhyacotriton olympicus*, 140-mm. *Cryptobranchus allegheniensis*, 72-mm. *Triturus viridescens*, and a 140-mm. adult *Gyrinophilus porphyriticus*.

Cytology. Although there is variation in the shape and structure of the ultimobranchial body, with respect to its cytological nature it is very similar in all of the species examined. The cells constituting this structure are derived from the pharyngeal epithelium and are at first rich in yolk. There is little cytoplasm; the nuclei stain intensely. Mucous

secretion is frequently formed in the cells found within the vesicles of the ultimobranchial body, as in the case of *Salamandra atra* (figs. 6 and 14). In *Amblystoma punctatum*, as well as in *Plethodon cinereus* where the cells exhibit more cytoplasm, the structure seems to be of a syncytial character. This, however, cannot be clearly demonstrated. There is, apparently, never any indication of degeneration. Mitoses are not frequent, although occasionally found. So far as this examination of the ultimobranchial body in the urodeles is concerned, no ciliated epithelium is found lining the cavities within it as Verdun ('98) notes in case of the duck.

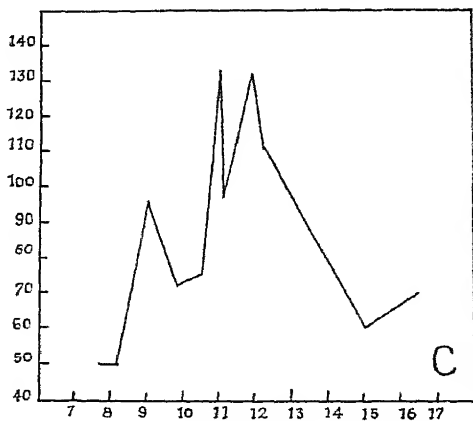
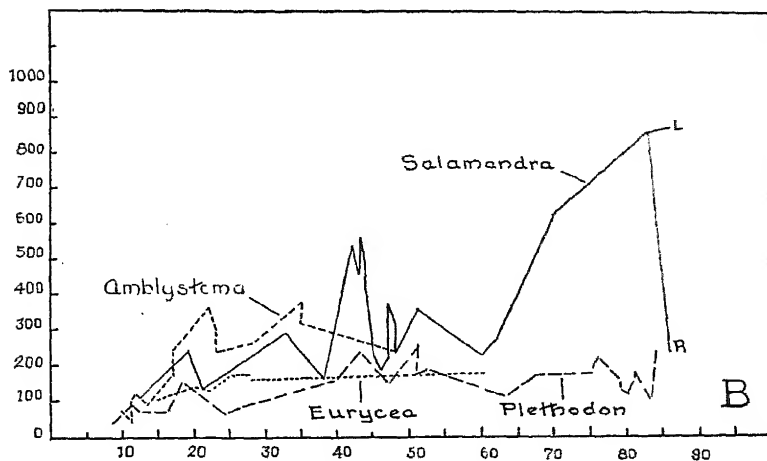
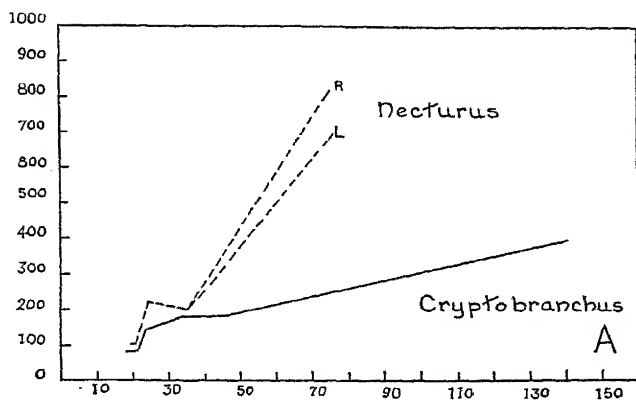
Size. In this study attention has been paid to the constancy of the ultimobranchial body with respect to size, and an attempt has been made to correlate this size with the total length of the individual. In his study of the growth of the thyroid and of the postbranchial body of *Amblystoma opacum*, Uhlenhuth ('24) states that the body length is more reliable than the total length and uses the former in expressing the weight of the organ relative to the size of the animal by the quotient $r^2 \frac{\text{weight of organ}}{\text{body length}}$ instead of $r^2 \frac{\text{weight of organ}}{\text{total length of animal}}$. Body length and total length he finds more significant than the weight of the animal. Against the total length of the animal, frequently the only dimension available in the specimens studied, the length of the ultimobranchial body in its cephalocaudal dimension has been plotted. A preliminary study made it seem desirable to use this dimension, and it is in this direction that the greatest increase in size takes place, probably because of the fact that in most cases it is situated between the *m. transversus ventralis*, and *m. thoracicohyoideus*, the pericardial cavity, and the cartilage of the branchial arch, all of which serve to limit its growth in other directions. Altogether, then, these measurements serve as criteria, not exact, but indicative rather of the general growth tendencies as the length of the animal increases, data being complete for but six individuals.

Of the Amblystomidae, one adult *Rhyacotriton olympicus* (fig. 22), as well as several individuals of all stages of

Amblystoma punctatum, were examined. In this specimen of *Rhyacotriton olympicus*, whose length is unknown, the ultimobranchial body is 580μ long. In the case of *Amblystoma punctatum* there is considerable variation in the size of the ultimobranchial body in the fifteen individuals noted in figure 2B, with variation to the extent of 120μ between 22-mm. and 23-mm. individuals. Growth of the structure apparently continues in older individuals, yet there is some evidence of a later decrease in absolute size, a decrease pointed out by Uhlenhuth ('24) as beginning with a "sudden drop during metamorphosis" and a "low relative weight during the rest of life." There is some variation in the length of the ultimobranchial body from those individuals of about the same size described by Baldwin. Baldwin found the ultimobranchial body to be about 300μ in a 40-mm. larva and 400μ in an early transforming stage, whereas I have found it to be at this stage 180, 195, 255, 300, and in one instance more than 890μ in length. In the late transforming stage he describes it (about 90μ long) as a "mass of poorly defined epithelial cells," stating that it is in the old adult entirely absent. In the head of an old adult examined by me the ultimobranchial body is present in fifteen sections.

Among representatives of the Salamandridae a 42-mm. larva of *Salamandra atra* possesses an ultimobranchial body 200μ long, relatively larger than in an adult specimen. A 23-mm. larval *Triton cristatus* possessed a non-vesiculated ultimobranchial body of 420μ , while in an adult (length unknown) it was more than 750μ long, the series being incomplete for the structure beyond this point. In the adult it is, moreover, vesiculated for 60μ at the anterior end and for 150μ at the posterior end, where 'colloid' is present in the

Fig. 2 Graphs showing the size (length) of the ultimobranchial body in proportion to body length in species of urodeles. The vertical line indicates length of the ultimobranchial body in micra; the horizontal, length of individuals in millimeters. A, *Necturus*, seven individuals examined; *Cryptobranchus*, six individuals. B, *Salamandra atra*, twenty-two individuals; *Amblystoma punctatum*, fifteen individuals; *Plethodon cinereus*, twenty-nine individuals; *Eurycea bislineata*, twelve individuals. C, *Hemidactylum scutatum*, fourteen individuals.



vesicles. In *Salamandra atra* data on the length of the ultimobranchial body and the total length of the animal in twenty-two individuals show the greatest increase in growth and the greatest variation in size to come at the time of metamorphosis, as may be seen in figure 2B, where individuals whose total length extends from 36 to 52 mm. show a variation ranging between 150 and 560 μ . Following this stage there is a gradual increase in size in the adult period. Two individuals, one 83 mm. and the other 86 mm., show the ultimobranchial body on the left side to be over 800 μ .

In a 14-mm. larva of *Desmognathus fuscus* the ultimobranchial body is over 70 μ . In a 15.5-mm. larva it is 60 μ , and is in this individual 56 μ in diameter at its anterior end, while it is about 8 μ in diameter as it extends laterad along the posterior wall of the last branchial afferent artery. In this specimen there is a similar strand of cells of the ultimobranchial body about 60 μ long and about 10 μ in diameter dorsal to the m. transversus ventralis. It is 90 μ long in a 16-mm. larva, between 100 and 160 μ in 18.5- and 10-mm. larvae. In one 30-mm. adult it is 80 μ long; in another animal of the same size it is 120 μ .

In the plethodontid *Gyrinophilus porphyriticus* the ultimobranchial body is relatively large, for in an 82-mm. larva (fig. 12), where there are but slight cavities, it is 420 μ long, being 140 μ in its greatest diameter, while in an individual measuring 95 mm. it is 720 μ , although here it consists of many tapering branches and is not compact. In an adult *Stereochilus marginatus* (fig. 17), where a very small cavity runs throughout its length, the body is 420 μ long.

Contrasted with these extremes in measurement is the size of the structure among individuals of *Plethodon cinereus*, *Batrachoseps attenuatus*, and *Eurycea bislineata*. In *Plethodon cinereus* it is 40 μ when first recognized in the embryo as a solid bud of cells pushing ventrally from the pharyngeal epithelium much as in *Hemidactylium scutatum*. In an embryo just ready to hatch it is 70 μ long. In a 10.5-mm. embryo it is 90 μ and here it is 100 μ in width, in this instance the

anteroposterior diameter not being the greater one. In a 19-mm. larva it is $150\ \mu$, while in a group of young adults ranging from 24 to 35 mm. the length of the body varies from 80 to $100\ \mu$ on the average, being still about $100\ \mu$ in its greatest diameter. The ultimobranchial body is extremely large in a 43-mm. adult, where it is $240\ \mu$ in its cephalocaudal dimension, yet in its longest extent it is about $280\ \mu$ and in its shortest about $100\ \mu$. Apparently, in *Plethodon cinereus* it is not the tapering structure that it is in other species, as in *Salamandra atra*, but is somewhat broader. In the group of older individuals ranging between 40 and 84 mm. the size of the ultimobranchial body varies between 150 and $180\ \mu$ on the average, although extremes show it to be $105\ \mu$ in an 83-mm. individual and $250\ \mu$ in a 51-mm. adult. In one adult *Plethodon cinereus* the ultimobranchial body is present on both sides (fig. 19), being $140\ \mu$ long on the right side and $240\ \mu$ on the left.

In *Batrachoseps attenuatus* the range in size of the structure is very similar to that in *Plethodon cinereus*. It is $40\ \mu$ long in a 12.5-mm. embryo, $100\ \mu$ in a 16-mm. larva, $50\ \mu$ in a 17-mm. larva, and $320\ \mu$ in an 85-mm. adult. An examination of larvae and adults of *Eurycea bislineata* shows very little difference in the size of the ultimobranchial body. A group of ten larvae between 20 and 31 mm. show the ultimobranchial body mostly a solid structure, being from 130 to $170\ \mu$ long and about $70\ \mu$ in its largest diameter, while in individuals between 30 and 60 mm. long it varies only between 150 and $180\ \mu$. Apparently, there is less tendency toward variation in size in *Eurycea bislineata* than in *Plethodon cinereus* and *Salamandra atra*. In these last two species, where the ultimobranchial body reaches an extreme size, secretion is present within the vesicles of the structure, especially in the case of the 70-mm. and 83-mm. adults of *Salamandra atra*; yet in an 86-mm. adult of this species where the ultimobranchial body reaches its maximum length there is none present.

Of the Sirenidae, two individuals of *Siren lacertina* and two of *Pseudobranchius striatus* were examined. In each of these species the ultimobranchial body was very different, both in structure and especially in size. While in one adult *Pseudobranchius striatus* it is $180\ \mu$ and in another $190\ \mu$ in length, it is relatively small in its other dimensions—which is not true of *Siren*, where the structure extends between the *m. transversus ventralis*, the *m. thoracicohyoideus*, and the blood vessel and is larger in all of its dimensions. In an adult (length unknown) it is similar, with its longest diameter extending laterad about $300\ \mu$, while its other diameter measures about $125\ \mu$. Here, as in *Plethodon cinereus*, it is apparently not attenuated, but is a broader, solid structure with its growth not confined to increase along an antero-posterior axis.

Asymmetry. Except in the case of *Necturus* noted by Platt ('96), the ultimobranchial body was stated by Maurer ('02) to occur on the left side only. This study confirms his generalization that the structure occurs "bei Urodelen meist nur linkseitig," although it was found that in *Amphiuma* means (fig. 18) it is a paired structure, as it is in *Necturus maculosus*. There are a few instances of its occurrence on the right side as well as the left, Baldwin noting this in a 19-mm. *Amblystoma punctatum* larva, but it is apparently never present on the right side alone. There is no evidence that the right body, once developed, ever degenerates.

In *Necturus maculosus*, as Platt described, the ultimobranchial body develops on either side in a symmetrical fashion. This symmetry I have found to be typical of the structure in all of the individuals examined and it is retained in the adult condition. Observations on its location, form, and size in *Necturus maculosus*, previously noted, have shown that in this species the structures assume a very symmetrical form (fig. 5). On either side they occur at approximately the same level and are usually of the same length, although, as has been noted, in one specimen the right body is $135\ \mu$ longer than the left.

In *Amphiuma means*, on the other hand, the right structure is chiefly anterior to the left, and in a young adult of 53 mm. the right body extends through sixteen sections, the left through eleven—making an exceptional instance in which the right structure has attained a larger size than the left. In *Amphiuma*, also, the caudal end of the right ultimobranchial body is usually on a level with the cephalic end of the left.

Another exceptional occurrence of a right ultimobranchial body occurs in an adult *Salamandra atra*. In this 86-mm. individual, the largest of this species examined, the structures on either side are to be found at the same level, but in this instance the left body is longer than the right, the left structure measuring 820 μ , the right 240 μ . The structure of the two is similar with respect to the appearance and size of the follicles (fig. 4).

An examination of more than fifty individuals of *Plethodon cinereus* shows but one adult (body length unknown) with the ultimobranchial body present on both sides (fig. 19), and here, as in *Amphiuma means*, the right is the more anterior, although not as long as the left, measuring 140 μ , the left 240 μ . The posterior 50 μ of the right structure is found at the same level with the anterior 50 μ of the left. Not only is the left structure longer, but it is much larger in its other dimensions.

In a 27-mm. larval *Desmognathus fuscus* the right ultimobranchial body is clearly present dorsal to the last branchial afferent artery, extending over 30 μ , while on the left the length is 90 μ . Although the ultimobranchial body of the right side was not in other instances clearly defined, there are sometimes groups of cells in characteristic position and grouping on the right side which indicate its presence. This is found to be true in a 17-mm. *Desmognathus fuscus* larva, an adult *Pseudobranchius striatus*, and a transforming *Salamandra atra* in which the gills are becoming shortened.

In *Necturus maculosus* the number of arches is reduced, there being four instead of five, as in other species of urodeles. In this species and in *Amphiuma means*, as well as

in those individuals of other species where it is exceptionally a paired structure, as mentioned, there is no indication of asymmetry in other structures in the region in which it is found, such as the muscles and blood vessels. It may be significant in this connection to note that the presence of a right ultimobranchial body occurring in those species where it is normally an asymmetrical structure is found in four families, i.e., the Salamandridae, Amblystomidae, Plethodontidae, and Sirenidae. It is therefore found not to be restricted to a particular group. The repetition of a symmetrical condition in the higher species of the urodeles would seem to be but a recurrence of a more primitive condition as evidenced in *Necturus*.

Development. The material available permitted a study of the development of the ultimobranchial body from early embryonic or larval stages to the adult in *Necturus maculosus*, *Cryptobranchus alleganiensis*, *Hemidactylium scutatum*, *Triturus viridescens*, *Desmognathus fuscus*, *Plethodon cinereus*, *Amblystoma punctatum*, and *Salamandra atra*. The earliest stage of development, a thickening of the epithelium of the pharynx, was observed in a 7-mm. *Hemidactylium scutatum*, an 11-mm. *Necturus maculosus*, an 8-mm. *Amblystoma punctatum*, and in embryos (length unknown) of *Triturus viridescens* and *Plethodon cinereus*. In these it first appears behind the last branchial arch as a thickening of the branchial endoderm on the ventral floor of the pharynx, later pushing vertically toward the dorsal surface of the pericardium as a solid bud of cells broadly joined to the epithelium. Baldwin (p. 640) has described this stage for *Amblystoma punctatum* in an 8-mm. larva.

Although not sufficiently well-defined to be identified certainly as the ultimobranchial body, a slight thickening of the branchial endoderm appears in an 11-mm. *Necturus maculosus* embryo, marking the site of the developing anlage. A 15-mm. embryo is the earliest form, as Platt ('96) also found, in which the ultimobranchial body is clearly recognizable. It is paired, as Platt found it to be, but is posterior to

the last gill cleft, instead of anterior to it as Platt stated. The connection with the branchial endoderm cannot be demonstrated, although the tapering dorsal end of each indicates a former connection. In a 16-mm. embryo it is very similar in structure on both sides, but there is a distinct cavity within it. Further vesiculation with irregular branching appears in a series of older embryos 20, 21, 23, 24, and 25 mm. in length, and this is also typical of the 70-mm. adult examined. The adult shows, as Platt has noted, that the bodies are still found near the place of their origin (fig. 5).

After the early stage—growth from the pharyngeal epithelium—the shape and position of the ultimobranchial body in the different urodeles markedly vary. Detached from the pharynx, it may consist of a finger-like structure, as in *Amblystoma punctatum* and *Salamandra atra*, or a compact oval body resting against the pericardium, as in a late larval stage of *Hemidactylium scutatum* (fig. 15) where the cells are typically grouped about a potential lumen.

A further variation in development appears in *Cryptobranchus allegheniensis* where, in the stage in which it is earliest recognizable, it exists as a ventral diverticulum in the position of a last pouch, undifferentiated in appearance from the pharyngeal epithelium and containing a very narrow lumen. As this becomes pinched off from the ventral side of the pharynx, it takes the form of a small vesicle and later becomes a pouch-like vesicle containing one large cavity.

Further differences in development and variation are due to the differences in the growth mechanics of the region. In the description of the location of the ultimobranchial body in the urodeles it was indicated that the development of the *m. thoracicohyoideus* has a great effect upon the form and position of the ultimobranchial body. Accordingly, the body may vary in its position from the median plane to close proximity to the cartilage of the last arch, and from immediately beneath the floor of the pharynx to the dorsal wall of the pericardium as a ventral limit.

Connection with the pharynx in early stages was observed in *Necturus maculosus*, *Salamandra atra*, *Hemidactylum scutatum*, *Triton cristatus*, *Triton alpestris*, *Triturus viridescens*, *Plethodon cinereus*, and *Amblystoma punctatum*. This connection is normally lost during early larval stages. However, in a *Triton alpestris* larva of 11 mm. the ultimobranchial body is connected with epithelium of the pharynx for one-half of its total length, and a connection persists in a *Triton cristatus* larva of 23 mm. and also was found in *Amphiuma* means of 30 mm. (fig. 13). The youngest larva of *Salamandra atra* examined, 12.5 mm. long, showed no connection of the ultimobranchial body with the pharynx.

Two unusual instances of a connection retained beyond the embryonic and early larval stages are found in *Salamandra atra* and in *Batrachoseps attenuatus*. In the former case a 45-mm. larva in the early transforming stage shows the ultimobranchial body broadly joined to the pharynx. There is no indication of such a retention of this embryonic condition of development in any of the other transforming larvae. In *Batrachoseps attenuatus* the retained connection with the epithelium of the pharynx occurs in an 85-mm. adult (fig. 21) and consists of a strand of cells, relatively slender compared with the broad connection just mentioned in *Salamandra atra*. In *Batrachoseps attenuatus* this constitutes a distinct exception to the usual development, for no connection is evident in 12.5-, 16-, and 17-mm. individuals. In the 85-mm. adult, where the entire structure is 320 μ in length, the connection persists for 160 μ . In both cases the form of the ultimobranchial body exhibits no other characteristics of an embryonic nature.

Vesiculation. The development of vesicles within the ultimobranchial body likewise bears no constant relation to the size of the animal. They tend to appear in relatively young larvae and continue to increase through metamorphosis, sometimes tending to increase as adult size is reached, sometimes showing a decrease. The development of such lumina within the body takes place at various times in different

forms. In *Necturus maculosus* this is first observed at 16 mm.; in *Plethodon cinereus* at 14 mm.; in *Cryptobranchus allegheniensis* at 18 mm., where a cavity is seen in the youngest individual in which the ultimobranchial body is formed. In *Hemidactylum scutatum* the lumen appears first in an 11.1-mm. larva. In *Salamandra atra* a 12.5-mm. larva (the youngest studied) has within its ultimobranchial body a distinct, relatively large lumen. In *Amblystoma punctatum* cavities first appear in a 23-mm. larva. Possibly there is a wider range of variation among other species in the age at which vesicles appear in the ultimobranchial body than their appearance in these species noted indicates.

Secretion. Demonstrable secretion is not commonly found within the vesicles of the ultimobranchial body of the urodele, although it is by no means unusual for it to occur. In some forms none can be recognized; in others it occurs in very slight amounts; in still others it is found closely resembling the colloid of the thyroid.

In most of the species where no secretion was found only a few individuals were examined. Inasmuch as only a relatively small proportion of the total number of individuals of all species examined exhibits secretion, it is not conclusively demonstrated from this study that there is no secretory activity within the ultimobranchial body of these forms. In *Amblystoma punctatum*, *Amphiuma* means, and *Triturus viridescens*, however, a large group of individuals was examined, and in these apparently there is none.

Secretion was noted in ten species of urodeles. A slight amount of secretion, together with cell remnants, is found within the lumina of the ultimobranchial follicles of *Necturus maculosus*, *Cryptobranchus allegheniensis*, *Hemidactylum scutatum*, *Eurycea bislineata*, *Typhlomolge rathbuni*, *Desmognathus fuscus*, and *Pseudobranchius striatus*. Traces of secretion are seen within the vesicles of a 70-mm. *Necturus maculosus*; in one vesicle on the right side the lumen is about one-quarter filled with it (fig. 16). A slight amount also is found in the one large regular ovoid vesicle present in the

ultimobranchial body of *Pseudobranchius striatus*, the same being true in the case of *Typhlomolge rathbuni*, where the one irregular vesicle just below the epithelium of the pharynx contains a similar amount of secretion. In two 41-mm. transforming larvae of *Eurycea bislineata* secretion is present, although not dense nor great in amount, while in a 60-mm. adult the vesicles are not large, their walls are thick, and occasionally within the lumina minute amounts of secretion are present. In a 30-mm. adult *Desmognathus fuscus* there are good-sized cavities in the ultimobranchial body, and within these there is secretion. Again, in a 35-mm. adult *Hemidaetylum scutatum*, where the ultimobranchial body is composed of three or four large branches, a slight trace of secretion is found within the cavity of one of these.

Secretion in such amount that it closely resembles the colloid of the thyroid follicles is found in the ultimobranchial body of *Triton cristatus* (fig. 7), *Salamandra atra* (fig. 14), and *Plethodon cinereus* (fig. 8). But one adult *Triton cristatus* was examined, and here the ultimobranchial body for a portion of its 750 μ is on a level with the thyroid, affording easy comparison with it (fig. 7). While the follicles are much smaller than those of the thyroid, they are of similar structure; the colloid stains identically with that of the thyroid. The fourth aortic arch intervenes between the two structures, the ultimobranchial body lying in its usual position dorsal to it, the thyroid being lateroventral to it. That this is not thyroid tissue is evident from its position, as well as from the difference in the size of the follicles in the two structures. It occurs here on the left side only.

Three specimens of *Salamandra atra*, a transforming larva of 43 mm., an adult of 70 mm., and one of 83 mm., show a considerable amount of secretion within the ultimobranchial body. In none of these does the secretion occur throughout the extent of the structure, but is restricted to one region which is enlarged to constitute a vesicle, as has been previously described. In the transforming larva it is clearly a mucous secretion which distends the follicle, the cells lining

it being greatly flattened (fig. 14). There is a very abundant vascular supply. In the two adults mentioned the secretion does not take the basic stain. The vascular supply is not so conspicuous in these, although capillaries are associated with them. In both of the adults secretion is found peripherally within the vesicle. Globules of such secretion in the epithelial lining in the adult may be seen in figure 6.

In *Plethodon cinereus* secretion is not localized in one region of the ultimobranchial body as it is in *Salamandra atra* and in *Triton cristatus*, but is found distributed in cavities within its different branches, although in some individuals one vesicle may be larger than the rest. A trace of secretion is found in the small, irregular vesicles of a 14-mm. larva and again in a 20-mm. adult, lying peripherally within the lumen of each in the middle of the structure. These are the youngest individuals in any species to show secretion. None, however, is found in any of the individuals ranging between 24 and 35 mm. In a 43-mm. specimen the characteristic vesicular structure is first apparent (fig. 3). The follicles from this stage on in the majority of individuals examined (fifteen out of twenty-three) contain secretion which typically fills them. In some individuals one follicle is much larger than the rest; in most individuals there are several smaller ones which may or may not contain secretion. In the adult where the structure occurs on the right as well as on the left the secretion resembles thyroid colloid.

In each of these species any great amount of secretory activity is attended by a good vascular supply. In *Cryptobranchus allegheniensis* this is apparent in a 140-mm. adult, where the pouch-like vesicle containing secretion has posteriorly a dorsal and a ventral prolongation. Between these two processes runs a blood vessel. At its posterior extremity it is encircled by a capillary plexus. In the older stages of *Plethodon cinereus*—between 43 and 90 mm.—capillary plexuses are closely associated with the ultimobranchial body. A specimen of *Plethodon glutinosus* also shows this particularly well. In one adult *Plethodon cinereus* the structure is very

unusually situated between three blood vessels, sending out slender processes between them.

From the taxonomic point of view it is interesting to note that, while in slight amount secretion is present in *Necturus maculosus* of the Necturidae, it is absent among those specimens examined of the Amphiumidae and Amblystomidae. Secretion in its greatest amount in any individuals is found in two European representatives of the Salamandridae, *Triton cristatus* and *Salamandra atra*, while several genera of the Plethodontidae exhibit it occasionally and some slight amount is found in *Pseudobranchius striatus* of the Sirenidae.

DISCUSSION

The point of greatest significance regarding the ultimobranchial body in the urodeles is concerned with its development, for its growth, location, and persistent occurrence in this group are distinctive. While in almost all of the species examined this pharyngeal derivative is an unpaired structure, it is for no apparent cause paired in two species of larger size, *Amphiuma* and *Necturus*, and occasionally paired in other species of smaller size.

Except in *Cryptobranchius allegheniensis*, the ultimobranchial body in all of the urodeles where its development is traced is formed, not as a pouch or as the derivative of a pouch, but as a thickening of the ventral entoderm of the pharynx. In *Cryptobranchius allegheniensis* the ultimobranchial body contains from the beginning a cavity continuous with that of the pharynx; the lumen in the adult structure is thus a portion of the embryonic pharynx. In all other urodeles in which development has been traced the developing structure, while continuous with the pharyngeal epithelium, consists of a narrow strand of cells. The connection is gradually constricted until complete separation is effected. There is no lumen in the connecting strand except in the instance mentioned, and all vesicles in the adult structure are secondarily developed.

The method of development in *Cryptobranchus allegheniensis*, however, which suggests the formation of a last branchial pouch, makes it appear somewhat more evident that the structure in the urodeles is derived from the branchiogenic region of the pharynx and is consequently 'ultimobranchial' rather than 'postbranchial.' This is in contradiction to Baldwin's ('18) observations and view (p. 641) in *Amblystoma*.

Irrespective of the varying number of pouches preceding it, the ultimobranchial body may be symmetrical or asymmetrical in its occurrence. Whenever asymmetrical, it occurs on the left side only. In a bird, the pied-billed grebe, according to Johnson ('20), the right ultimobranchial body atrophies, but such is not the case among the urodeles, where the asymmetry is due to a complete lack of development on the right side. No reason can at present be given either for the absence of a right ultimobranchial body or for its sporadic occurrence in a number of different species.

The universality of the presence in the urodeles of this distinctive structure suggests, at first, that it must have some physiological significance. Indication of a secretory activity is, however, slight and in only a few instances would seem to be of significance. In the urodeles the ultimobranchial body contains, in general, a slight amount of secretion and this not of uniform occurrence.

However, the possibility that the ultimobranchial body possesses a glandular nature similar to that of the thyroid may be considered, since its relationship to the thyroid gland has been suggested from the time of its earliest description.² Uhlenhuth and McGowan, in considering the ultimobranchial body and the thyroid together in *Amblystoma opacum*, find

² De Meuron's ('86) study of the ultimobranchial body in the Amphibia led him to consider them as 'accessory thyroids,' since the bodies come to lie so near the thyroid. Maurer objected to this nomenclature, since he found the 'postbranchial' bodies in amphibians in no way resemble the thyroid. As Camp ('17) points out, Maurer believes that De Meuron described correctly the origin of the 'postbranchial' bodies, but that he has confused their later development with the epithelial derivatives of the pouches, which come to lie near the thyroid.

that the 'postbranchial' body is of little physiological significance. They do not correlate any activity on its part with metamorphosis; rather do they find it inactive at this time. Although he makes no correlation between the two structures in *Typhlomolge rathbuni*, Uhlenhuth ('23) does note that the 'postbranchial' body in this species "shows sometimes signs of developmental inhibition," while but rudiments of the thyroid are present in six specimens and in a seventh the thyroid is entirely absent. The 'postbranchial' body in some animals he finds reaching back to the heart and attached to the pericardium; he states that "its posterior end, however, does not attain the size which this part is found to attain in *Ambystoma*." Like Uhlenhuth, I have found, in the one specimen examined, that the structure "resembles much that of other salamanders, but sometimes is shorter and lacking a lumen." In these cases he states that "the organ remains short, extending backward only to the middle between pharynx and pericardium." While the structure, as I have found it, is similar to those which he describes in each instance as "an epithelial structure of the shape of a tube possessing, in places, epithelial diverticula," it does not, however, connect with the pharyngeal epithelium, as in the specimens he describes, although it is situated very close beneath it.

Although the ultimobranchial body is quite separate from the thyroid, it exhibits in some of the urodeles a certain resemblance to thyroid in that epithelial vesicles occur which contain 'secretion' of a colloidal nature. This was particularly true of *Triton cristatus* (fig. 7), as has been already noted. However, since the colloidal character of the 'secretion' is not usually demonstrable, and the existence of the epithelial vesicles themselves is frequently lacking, it is obvious that the resemblance to the thyroid is purely superficial. It should, of course, be appreciated that any secretion retained within a closed space tends to become colloidal in character through reabsorption of water.

While, in the mammals, the developmental relation of the ultimobranchial body and the thyroid becomes very intimate, there even being evidence, as previously noted, that the ultimobranchial body differentiates into colloid-filled vesicles, in birds and lower vertebrates generally, no such transformation to thyroid tissue occurs.

The ultimobranchial body of urodeles thus presents no uniform picture of secretory activity. Not only are there marked differences in different species, but within the species striking individual differences occur. *Plethodon cinereus*, among the species examined, presents a greater uniformity in this respect. The evidence gained from an examination of the extensive material made use of in this investigation is purely negative; there is afforded no support for the assumption that the structure in question represents an internally secreting gland, nor is there any indication that it represents an exocrine gland that has lost its duct, since there is never among the urodeles any indication of a duct leading from the ultimobranchial body to the pharynx, such as Verduin noted in the duck where a tube of ciliated epithelium connects the ultimobranchial body with the pharyngeal epithelium, or as in the case of *Squalus acanthias* where Camp notes that "a part of the gland in the adult may secondarily become connected with the pharynx by a true duct." If not a gland, there remains the interpretation offered by Kingsbury, already referred to (p. 286).

Whatever the significance of the ultimobranchial body, morphologic or physiologic, the observations in this article point to the fact that this structure occurs without exception in a large number of salamanders, and probably in all. In general, these observations agree with the descriptions of the ultimobranchial body in those urodeles, previously listed, in which an examination had already been made. Certain points of difference occur in connection with the general statement of Maurer's concerning the type of secretion found within its vesicles, the statement of Baldwin's concerning the absence of the structure in the heads of old adults of *Amblys-*

toma punctatum, and the description of the location and development of the ultimobranchial body in *Necturus* as described by Platt. Contrary to Platt's ('96) description of the position of the bodies anterior to the last gill cleft, I have found them occurring posterior to the last arch, and from this they may be regarded, as in every other form, 'ultimobranchial.'

Reviewing the characteristics of this curious development from the urodele pharynx as determined by a survey of extensive material, the outstanding feature is its marked variability. This variability applies to position, form, structural differentiation, and indications of a secretory activity or lack of it. The differentiation of its cells is frequently so slight that there is the suggestion of a persistence of an essentially embryonic condition.

SUMMARY

The significance of the ultimobranchial body has been the object of this comparative study of the structure in the following species of urodeles:

<i>Necturus maculosus</i>	<i>Hemidaetylum scutatum</i>
<i>Typhlomolge rathbuni</i>	<i>Plethodon cinereus</i> and <i>P. glutinosus</i>
<i>Amphiuma means</i>	<i>Stereochilus marginatus</i>
<i>Cryptobranchus alleganiensis</i>	<i>Gyrinophilus porphyriticus</i>
<i>Triturus torosus</i> and <i>T. viridescens</i>	<i>Pseudotriton ruber</i>
<i>Triton cristatus</i>	<i>Eurycea bislineata</i>
<i>Triton alpestris</i>	<i>Desmognathus fuscus</i> and <i>D. ochrophaeus</i>
<i>Salamandra atra</i>	<i>Typhlotriton spelaeus</i>
<i>Amblystoma punctatum</i>	<i>Siren lacertina</i>
<i>Rhyacotriton olympicus</i>	<i>Pseudobranchius striatus</i>
<i>Batrachoseps attenuatus</i>	

In nineteen of these twenty-four species it has not hitherto been described.

Caudal to the last branchial arch, it develops as a thickening and later as an outpushing from the ventral wall of the pharynx. Due to the growth mechanics of the region, it comes to lie obliquely to the pharynx, ventral to it and dorsal or dorsolateral to the pericardial cavity in its anterior region. It persists throughout life as an epithelioid or epithelial

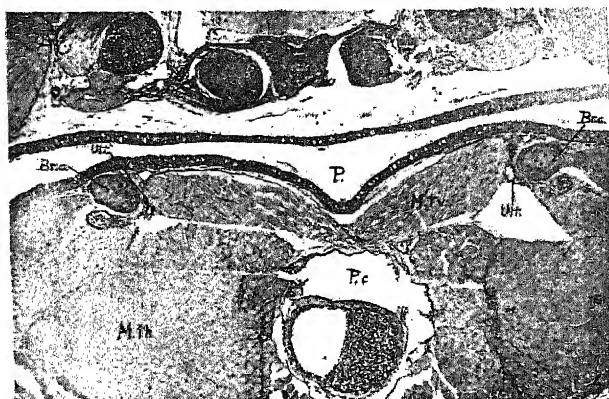
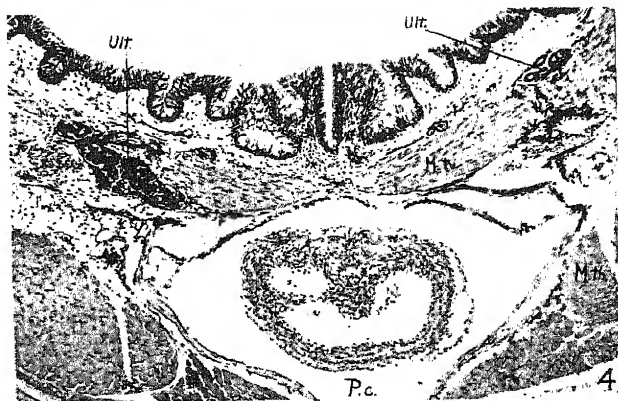
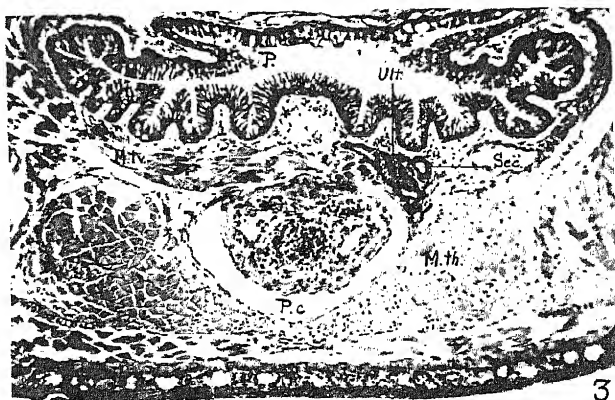
structure, usually of irregular shape, frequently containing vesicles; in some cases it exhibits a considerable amount of secretory activity of variable quality. Except in *Amphiuma* and *Necturus* where it is regularly paired and in occasional instances in individuals of other species where it occurs on both sides, it is usually present on the left side only. Its occurrence is constant in all of the species of urodeles for which it has been examined.

It is variable in size, form, and position. This, together with the quite inconstant indication of secretory activity, marks it as a structure of little or no physiological significance. 'Colloid' is, however, present in some instances, and hence a comparison with the thyroid was considered.

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ABBREVIATIONS

<i>A.a.</i> , last aortic arch	<i>P.</i> , pharynx
<i>A.l.</i> , aditus laryngis	<i>P.C.</i> , pericardial cavity
<i>Br.c.</i> , cartilage of last branchial arch	<i>Sec.</i> , secretion
<i>Cap.</i> , capillary	<i>Thy.</i> , thyroid
<i>M.th.</i> , m. thoracicohyoideus	<i>Tr.a.</i> , truncus arteriosus
<i>M.tv.</i> , m. transversus ventralis	<i>Ult.</i> , ultimobranchial body

PLATE 1

EXPLANATION OF FIGURES

3 Photograph of a transection through the heart of a 43-mm. *Plethodon cinereus*, showing the relation of the ultimobranchial body to the pericardial cavity, pharynx, m. transversus ventralis, and m. thoracicohyoideus. The vesicles here are filled with faintly staining secretion. $\times 56$.

4 Photograph of a transection through the heart region of an 86-mm. *Salamandra atra*. The ultimobranchial body is paired in this individual. $\times 30$.

5 Photograph of a transection through the heart and ultimobranchial bodies of a 70-mm. *Necturus maculosus*, showing the ultimobranchial body in a paired condition lateral to the m. transversus ventralis, between this and the cartilage of the last branchial arch, and relatively near the pharynx. $\times 22\frac{1}{2}$.

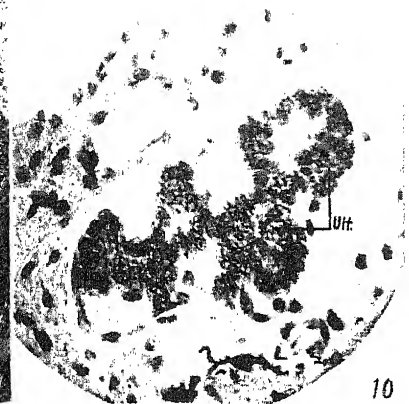
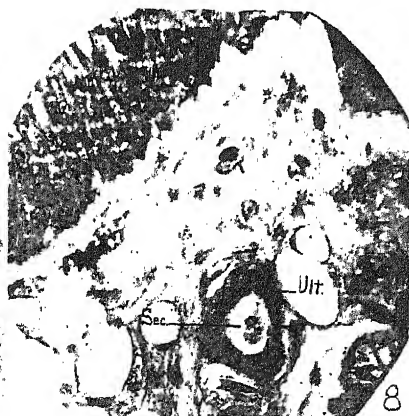
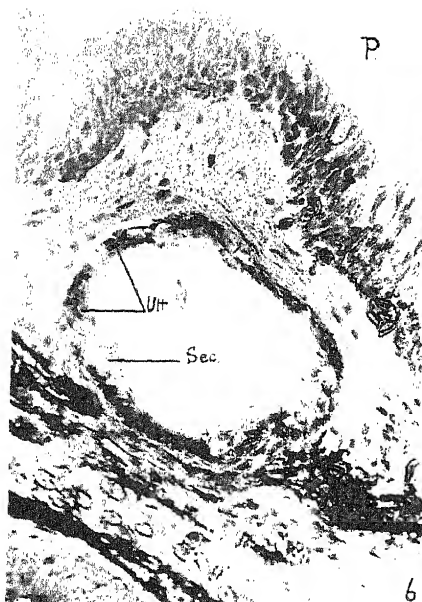


PLATE 2

EXPLANATION OF FIGURES

6 Photograph of a transection through the ultimobranchial body of a 70-mm. *Salamandra atra*, showing one large follicle beneath the epithelium of the pharynx. Secretion is found within it, but, unlike that in figure 14, it is chiefly peripheral. $\times 135$.

7 Photograph of a transection through the ultimobranchial body and thyroid of an adult *Triton cristatus*. The structure of the follicles is similar, although there is a characteristic difference in their size. The secretion found within the ultimobranchial body gives a staining reaction identical with that of the thyroid colloid. The last aortic arch intervenes between the follicles of the thyroid and those of the ultimobranchial body. $\times 67\frac{1}{2}$.

8 Transection through the ultimobranchial body of a 75-mm. *Plethodon cinereus*, showing secretion within the one large follicle which composes the structure. This is typical of the structure in many adults. $\times 240$.

9 Transection through the ultimobranchial body of a 67-mm. *Plethodon cinereus*. The inner surface of the one large follicle composing the structure resembles that of a thyroid follicle. $\times 240$.

10 Transection through the ultimobranchial body of an adult *Plethodon cinereus*, showing another characteristic form of the adult structure in this species. Here the ultimobranchial body is very irregular, less compact than those shown in figures 8 and 9, and contains several follicles. $\times 240$.

PLATE 3

EXPLANATION OF FIGURES

11 Photograph of a transection through the aditus laryngis (at the left) and the ultimobranchial body of a 133-mm. *Siren lacertina*, showing the way in which the position and form of the ultimobranchial body is dependent upon the development of the muscles between which it is found. Here it is shown in contact with the last aortic arch. $\times 67\frac{1}{2}$.

12 Photograph of a transection through the aortic trunk and ultimobranchial body of an 82-mm. larval *Gyrinophilus porphyriticus*, showing the ultimobranchial body on the left side in contact with the last aortic arch and between the m. transversus ventralis and m. thoracicohyoideus. $\times 67\frac{1}{2}$.

13 Photograph of a transection through the aditus laryngis and the ultimobranchial body of a 30-mm. *Amphiuma means*. The section shows the relation of the ultimobranchial body, at this stage connected with the pharyngeal epithelium, midway between the cartilage of the last branchial arch and the aditus laryngis, lateral to the m. transversus ventralis and extending ventrally nearly to the pericardium. $\times 135$.

14 Photograph of a transection through the ultimobranchial body of a 43-mm. *Salamandra atra*, showing a large follicle containing mucous secretion. The secretion nearly fills the follicle, which occupies the usual position for it in this species. $\times 67\frac{1}{2}$.

15 Photograph of a transection through the ultimobranchial body of a 16.5-mm. *Hemidactylum scutatum*, showing the rather hemispherical shape of the structure in cross-section. Connection with the pharynx has been lost, and the ultimobranchial body is found in contact with the dorsal surface of the pericardial cavity. $\times 135$.

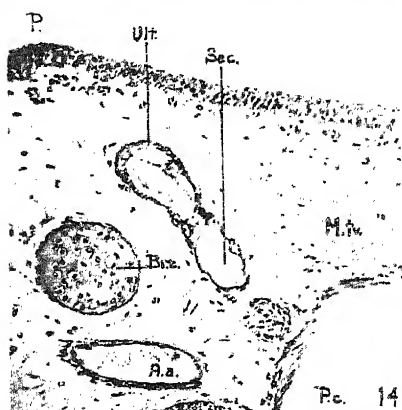
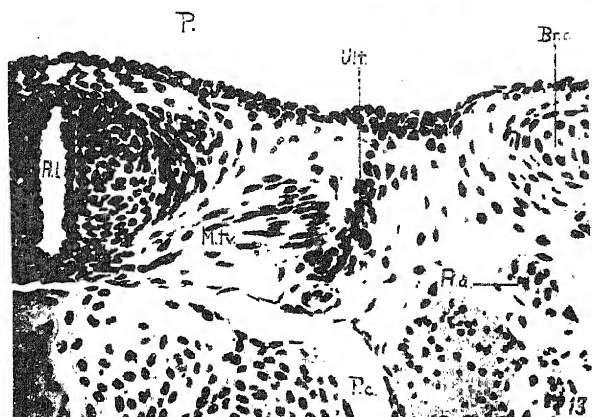
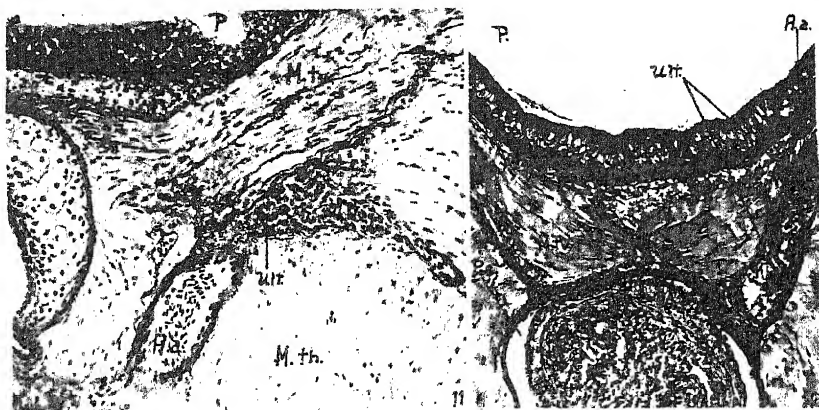


PLATE 4

EXPLANATION OF FIGURES

16 Photograph of a transection through the right ultimobranchial body of a 70-mm. *Necturus maculosus*. The ultimobranchial body is wedged between the cartilage of the last branchial arch and the m. transversus ventralis. In the most dorsal vesicle secretion is visible. $\times 135$.

17 Photograph of a transection through the heart and ultimobranchial body of an adult *Stereochilus marginatus*, showing the proximity of the ultimobranchial body to the pericardial cavity. This section is through an unusually long transverse process of the ultimobranchial body. $\times 67\frac{1}{2}$.

18 Photograph of a transection through the heart and ultimobranchial bodies of a 33-mm. *Amphiuma means*. In this species the ultimobranchial body is normally paired as in *Necturus maculosus* (fig. 5); apparently it is more symmetrical in these forms than in *Salamandra atra* (fig. 4), where the pairing is a very unusual feature. $\times 56$.

19 Photograph of a transection through the posterior pharyngeal region of an adult *Plethodon cinereus*, showing the ultimobranchial body present on both sides. As in others of this species, the structure is anterior to the pericardial cavity. $\times 52\frac{1}{2}$.

20 Photograph of a transection through the ultimobranchial body of a 32-mm. *Salamandra atra*, showing the intimate relationship of the capillary supply from the last aortic arch. The structure is situated near the dorsolateral surface of the pericardial cavity. $\times 262\frac{1}{2}$.

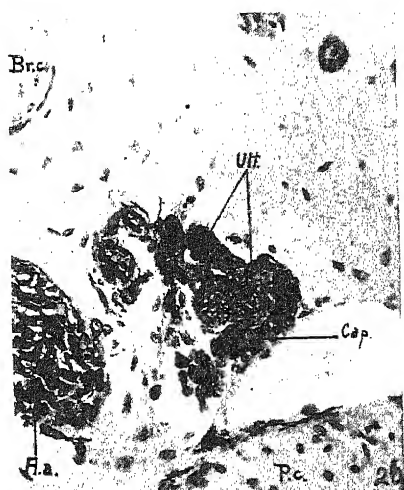
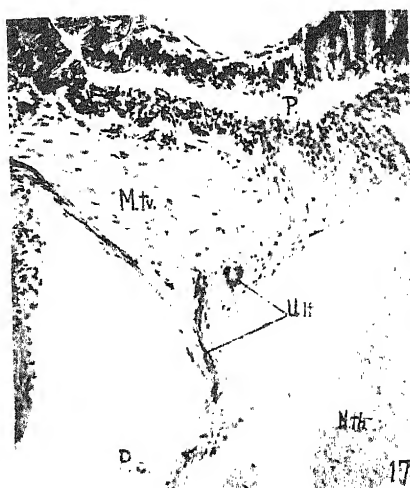
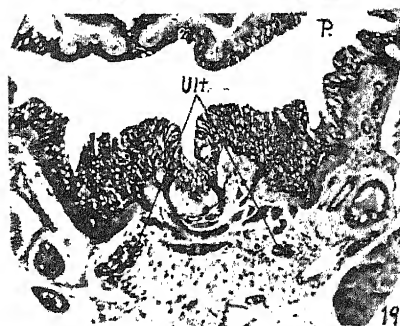
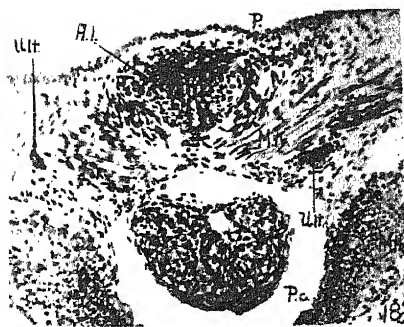
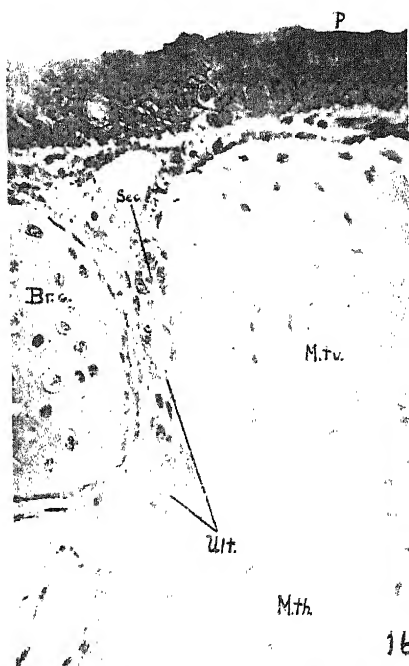


PLATE 5

EXPLANATION OF FIGURES

21 Photograph of a transection through the posterior region of the head of an 85-mm. *Batrachoseps attenuatus*, showing the connection of the ultimobranchial body with the epithelium of the pharynx beyond the time when it is normally separated from it. This section is anterior to the pericardial cavity, passing through the branches of the truncus arteriosus. $\times 67\frac{1}{2}$.

22 Photograph of a transection through the pharynx and ultimobranchial body of an adult *Rhyacotriton olympicus*, showing the dorsoventral flattening of the ultimobranchial body and its position dorsal to the m. thoracicohyoideus. Branches of the last aortic arch are in contact with it. $\times 67\frac{1}{2}$.

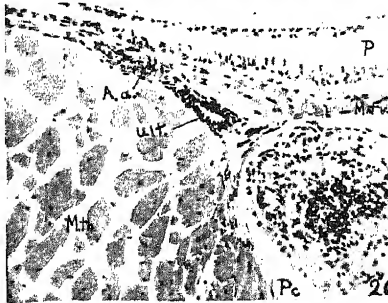
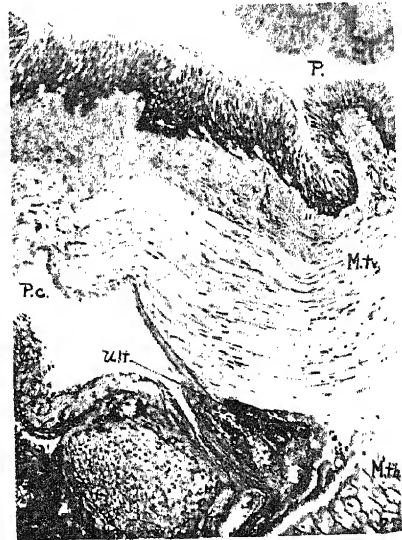
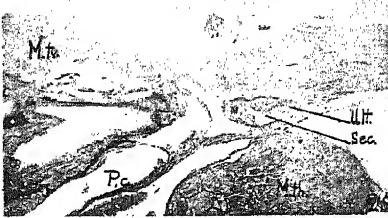
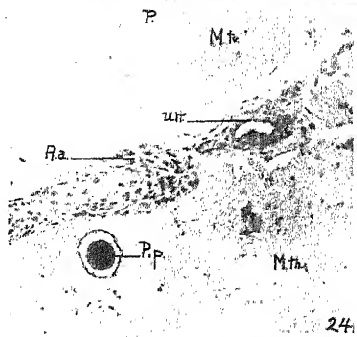
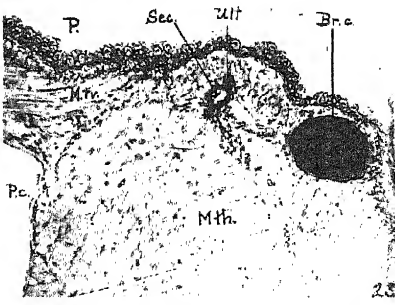
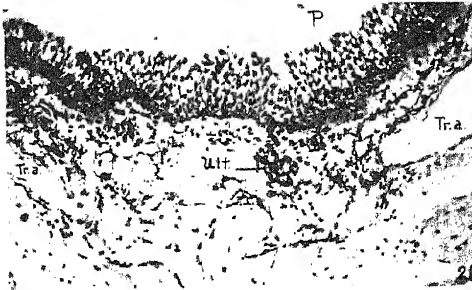
23 Photograph of a transection through the pericardial cavity and ultimobranchial body of a 45-mm. *Cryptobranchus alleganiensis*, showing secretion within the single vesicle of the ultimobranchial body. The ultimobranchial body is found near the pharynx, due to the dorsal position of the extremely large m. thoracicohyoideus. $\times 67\frac{1}{2}$.

24 Photograph of a transection through the posterior region of the head and through the ultimobranchial body of an adult *Pseudobranchius striatus*, showing the one larger vesicle of the ultimobranchial body in this specimen closely applied to the wall of the last aortic arch and far removed from the pericardial cavity. As in figure 10, it is between the two muscles. The m. thoracicohyoideus is seen to be heavily parasitized with an undetermined species of parasitic Protozoa. $\times 67\frac{1}{2}$.

25 Photograph of a transection through the heart and ultimobranchial body of a 30-mm. *Desmognathus fuscus*, showing the position of the ultimobranchial body beside the pericardial cavity, ventral to the m. transversus ventralis and dorsal to the m. thoracicohyoideus. The vesicle contains secretion. $\times 135$.

26 Photograph of a transection through the pericardial cavity and the ultimobranchial body of an adult *Eurycea bislineata*, showing the relations of the ultimobranchial body to the muscles and the last aortic arch similar to those found in figure 7. It extends along the last aortic arch from the pericardial cavity nearly to the pharynx. Right and left sides are here reversed. $\times 67\frac{1}{2}$.

27 Photograph of a transection through the pericardial cavity and ultimobranchial body of an adult *Typhlotriton spelaeus*, showing the typical position of the ultimobranchial body between the m. transversus ventralis, the m. thoracicohyoideus, and the pericardial cavity. $\times 67\frac{1}{2}$.



THE COMPARATIVE ANATOMY OF THE LIPS AND LABIAL VILLI OF VERTEBRATES

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FIVE TEXT FIGURES AND NINE PLATES

AUTHOR'S ABSTRACT

An attempt is made to define lips, and on Danforth's interpretation of homology, homologous lips are found at certain stages of development in some representatives of all classes of vertebrates. The primary lips characteristic of selachians, after the maxillary and premaxillary bones have developed within the territory of the upper lip (toadfish, cod), may disappear (trout, *Spelerpes*), accompanied by a forward migration of the lower jaw. The secondary lips of higher forms are first indicated in certain teleosts and amphibians. Lips vary in structure to accord with their physiological functions, whether sensory, prehensile, or adhesive. Lips of the cod are highly sensory; those of the tadpole and of grazing animals, in different ways, are notably prehensile; the lips of *petromyzon* and the vampire, having abundant villi, are most effectively adhesive. Therefore, the smaller villi of the lips of suckling animals are presumably for tight adhesion to the nipple. The opossum and rat, however, nurse before their lips have developed. No free macroscopic villi are found on human lips, but there is a zone of thick epithelium tending to form villi. Such a zone is shown to be a widespread feature of vertebrate lips.

INTRODUCTION

At the meeting of the American Association of Anatomists in 1924, Dr. F. T. Lewis described the nasomaxillary angles of human lips, both embryonic and adult, and suggested that the transient labial villi found in tiers between the teeth and the skin might represent abortive generations of primitive teeth. Interested in determining further the real significance of these peculiar villi, he proposed that I should make a comprehensive study of the problem, which has been done, under his general supervision, during the four years that I have held a National Research Council Fellowship at the Harvard Medical School.¹ All reasons for regarding the villi as denticles have been presented in an earlier paper, in which the

¹ The preparation of manuscript and drawings for publication was continued at Northwestern University, where Professor Arey provided technical and other assistance which is gratefully acknowledged; Miss M. Walsh drew many of the figures included in plates 1, 4, and 6.

remarkable tooth-like processes within the upper lip of the cat, mimicking on a small scale the forms of selachian teeth, are described in detail apparently for the first time. My conclusion then was that a first step had been taken in establishing the 'denticle' interpretation, but that a further one was needed, and it was stated that a study for that purpose was in progress. This has now been completed, and any relationship between villi and ancestral teeth might be definitely denied were it not for the iconoclastic interpretation of cranial homologies earnestly advocated by Kesteven in a long series of papers. Probably Professor Starks is in accord with most authorities when he comments on Kesteven's work²—"His view, ingenious as it is, I do not find myself able to accept." Were it correct, a most interesting relationship between the villi and the premaxillary teeth of teleosts might exist, as will be shown incidentally in its proper place. Our primary interest, however, is in the comparative anatomy of the lips, with a view to interpreting their conditions in man. But who knows what a lip is, or which vertebrates have them?

Meckel has been the guide and leader of the German anatomists in this discussion.³ In 1811, he wrote:

Die Lippen fehlen den meisten Säugthieren und allen tiefer als sie stehenden Wirbelthieren, erscheinen aber auch bei dem menschlichen Embryo nicht vor Ablauf der ersten beiden Monate nach der Empfängniss.

Little by little the German writers have admitted a wider occurrence of lips. Wiedersheim, in early editions of his Lehrbuch,⁴ emphasized the muscular element as essential to true lips, and saw in mammals "hier zum erstenmal auftretenden eigentlichen Lippenbildungen." But, somewhat more lenient than Meckel, he adds: "Die Cetaceen und Monotremen sind die einzigen Säugethiere, welche der Lippenbildun-

²In a personal communication.

³Meckel, J. F. *Beyträge zur vergleichenden Anatomie*, Bd. 2, Heft 1, Leipzig, 1811, S. 45.

⁴Wiedersheim, R. *Lehrbuch der vergl. Anat. der Wirbelthiere*, 2. Auflage. Jena, 1886, S. 482.

gen gänzlich entbehren." Kükenenthal was astounded by this statement, in so far as it refers to Cetacea.⁵ He writes:

Diese Bemerkung Wiedersheim's ist um so unverständlicher, als ja gerade bekanntermaassen bei den Bartenwalen die Erhebung der Unterlippe eine ganz ausserordentlich grosse ist und bei der Nahrungsaufnahme eine wichtige Rolle zu spielen hat.

And he quotes Eschricht, "schon vor fast einem halben Jahrhundert," for details. Kopsch, in Rauber's Lehrbuch, does not heed this correction,⁶ but Wiedersheim has done so, and the last word on the subject, from Schumacher,⁷ is as follows:

Eigentliche, d. h. mit Muskulatur versehene, Lippen finden sich erst bei den *Säugetern*. Die „Lippen“ bei *Fischen* dürfen nicht mit den muskulösen Lippen der Säugetiere homologisiert werden; sie stellen nur Hautfalten mit senkrecht sich überkreuzenden Bindegewebszügen dar (OPPEL). Die fleischigen Lippen der Säugetiere, in Gemeinschaft mit den Backen sowie mit der beweglichen, muskulösen Zunge, ermöglichen das Saugen und stehen auch in wichtiger Beziehung zur artikulierten Sprache des Menschen. Den *Monotremen* fehlen Lippenbildungen.⁸

Thus the German anatomists who see lips as primarily a muscular suckling apparatus, consistently restrict them to mammals; but others who consider that lips devoid of muscle would still be lips, find them more widely distributed. We shall see that, in the nursing stage, the lips of mammals are very often but slightly developed.

⁵ Kükenenthal, W. Vergl.-anat. und entw. Untersuchungen an Walthieren. Th. 2, Jena, 1893. Denkschr. d. med.-naturw. Ges. zu Jena, Bd. 3, S. 317.

⁶ "In der Tierreihe kommen Lippen nur den Säugetieren zu, doch fehlen sie den Monotremen und Walen." (Rauber's Lehrb. d. Anat. d. Mensch., 7. Aufl., Abt. 4, 1907, S. 15).

⁷ Hdb. d. mikr. Anat. d. Mensch., herausgegeben von v. Möllendorff, Bd. 5, Teil 1, Berlin, 1927, S. 13.

⁸ Most of this passage is an unacknowledged quotation from Wiedersheim. But Keibel independently prefers to restrict the term lips to the structures in man and mammals. "Ich selbst ziehe es vor, den Ausdruck Lippen für die fleischigen Hautmuskelwülste zu reservieren, welche den Mund der Säuger und des Menschen umgeben" (Hertwig's Handb. d. vergl. u. exp. Entw. d. Wirbeltiere, Bd. 1, Teil 2, 1906, S. 156.)

A very different position has been taken by French and English anatomists.⁹ Cuvier, while admitting that many sorts of fish are lipless, found that more often their mouths are bordered with soft and extensible lips, sometimes 'très grandes' or 'très prononcées.' Among amphibians "la bouche est bordée de lèvres dans la *sirène* et le *protée* . . . La peau forme autour de la bouche (des *amphiuma*) des lèvres très marquées." Although Cuvier found no mobile lips in reptiles, and only so-called lips in birds, he recognized their presence "dans tous les mammifères, sauf dans les échidnés. . . . Dans l'*ornithorhynque* elles n'ont aucune mobilité."¹⁰

Similarly, Owen, who knows that fishes "have not so distinct a *sphincter oris* as mammals," ascribes to some of them 'thick fleshy lips.' In reptiles—Ophidia, Sauria, and Crocodilia—

a narrow tract of soft and vascular integument intervenes between the scale-clad border of the mouth and the jaws, sinking into a more or less shallow groove which defines the lips and receives the secretion of a row of mucous crypts: but such lips are hard and inflexible: in certain frogs and toads they are of softer texture: but in none are produced or prehensile.¹¹

With this Göppert agrees, differing from the prevalent opinion of German anatomists when he writes:

Lippen besitzen ferner die Mehrzahl der Reptilien. Sie beherbergen hier die Glandulae labiales. Ihr Fehlen bei den Cheloniern erklärt sich aus einer Rückbildung. . . . Bei den Säugetieren . . . durch den Besitz einer Muskulatur stehen sie auf besonders hoher Entwicklungsstufe.¹²

⁹ Compare Rondeletius, G., *Libri de piscibus*, Lugduni, 1554, Lib. III, Cap. 8, p. 57: "Whoever credulously believes that there are no lips in fishes will at once change his mind when he sees and handles rock-fishes, which have fleshy, true and perfect, mobile lips. . . . Very many fishes then lack lips: some have them, like the rock-fishes which browse on moss, and take their food with lips instead of hands, as oxen, sheep and the like, which live by grazing; and they must have lips since their tongue is imperfect." (Note by Dr. Lewis.)

¹⁰ Cuvier, G. *Leçons d'anatomie comparée*. 2. éd. T. 4, part. 1, Paris, 1835, pp. 381-409.

¹¹ Owen, R. *On the anatomy of vertebrates*, vol. 1, London, 1866, pp. 410-411; 434.

¹² In Hertwig's *Handbuch*, Bd. 2, Teil 1, 1906, S. 79.

Rejecting facial musculature as the essential feature of lips, they may be defined as fleshy folds bordering the entrance to the mouth, placed anterior to the skeletal supports of the jaws, and separated from the dentigerous zone by a groove, the sulcus labialis or vestibulum oris; lips may be partially supported by labial cartilages, and may be rendered mobile through the invasion of skeletal muscle. In man the lips possess two zones, described by Luschka as the pars villosa and pars glabra, and after a lapse of some years these terms and the parts which they stand for have become quite familiar. It is, however, a question whether in the human lip these villi are distinct and separate in life, or whether tall connective-tissue papillae extend into a very thick epithelium, which through easy maceration becomes cleft to form the villi. Though the pars villosa is a very distinct zone in the lips of newborn infants, separate villi are not ordinarily seen, even with a hand lens. Rounded or, at best, conical papillae were observed by Dr. Lewis in the untreated lips of a dead infant kept a short time in cold storage, but the same specimen after a night in Ringer's solution, or after hardening and shrinking in Zenker's fluid, showed separate villi very clearly. He has called my attention to the statement by Ruysch (*Thesaurus anatomicus* vii, No. xl, Not. 3) that "in the lips the papillae do not come into view *unless the epithelium has first been removed*, whereas in the intestine elevations in the form of silky hairs (villi) are seen like a fleece *without ablation of the integument or epithelia*." "As to function," Ruysch continues in Not. 5, "I think that the papillo-nervous body is an instrument of sensation (sensus) and hence such fierce pain where, by humors, bitter medicines or other things, the epithelium has been removed, so that the papillae are affected." There is a measure of truth in this statement, and a valid criticism of some more modern accounts, but a discussion of these details finds its place in a final section of this paper.

True lips, then, appear to be essentially protrusible folds in front of the teeth, over which the skin connects with mucous membrane, and on the surface of which two zones may be

distinguished—an outer smooth zone and an inner papillary ('villous') zone, perhaps sensory in function. The animals studied in search for lips to be recognized by this new criterion will be presented in the conventional zoölogical order. For this investigation 302 sagittal series in the Harvard Embryological Collection were utilized, together with 162 partial series specially prepared for the purpose. Either in the gross or with the binocular microscope, 825 whole specimens were examined. The figures accompanying this paper were selected from 900 tracings and 228 photographs or sketches collected during the progress of the work.

CYCLOSTOMES

Unfortunately, we begin with an aberrant form, the hypophysis of which, instead of remaining in the roof of the mouth, migrates to the top of the head. Were it considered an essential landmark, lips, if present, would be in no relation with the mouth. This difficulty has been considered by His, who proposed to place the lips of *Petromyzon* in a class by themselves, naming them 'Rachenlippen'; but Keibel, who has arranged an interesting series of embryonic sections of petromyzons and rabbits to illustrate this feature, would not call them lips at all.¹³ It becomes a question as to whether the hypophysis is an all-important landmark in this orientation. After it has migrated beyond the oral territory, the mouth becomes overhung by a hood which in descriptive literature is often called a lip. Thus Dohrn ('82), when writing of larval lampreys, refers to the 'extraordinary growth of the upper lip' which he figures together with the 'Unterlippe.'¹⁴ For this he is criticised by His ('92) for the obvious reasons already noted, which were of course well known to Dohrn.

¹³ This is in Keibel's important chapter dealing in a general way with the entire subject of our thesis (Kap. 6 in Hertwig's Handbuch, Bd. 1, Teil 2, 1906, S. 156 and 158). See also His, Arch. f. Anat. u. Entw., 1892, S. 410.

¹⁴ Mitth. d. zool. Stat. zu Neapel, Bd. 4, S. 177.

Prominent in larval stages, the hood or upper lip later retracts, and in continuity with the fold which represents a lower lip it bounds the funnel or round mouth of the cyclostomes. In relation with the fleshy circumference of this suctorial mouth, the much-discussed horny teeth are developed and, in front of them, a fringe of tentacles possibly comparable with villi. In greater detail the development of the mouth may be described as follows.

In an embryo of 1.8 mm. the pharyngeal cavity has formed, but there is only a slight corresponding invagination of the ectoderm. In the 2.7-mm. embryo (fig. 6) the anterior extremity of the foregut has encountered the ectoderm at the bottom of a considerable depression, deeper, in fact, than is usually found in the stomodaeum of vertebrates. Just in front of the ectodermal cavity there is a hypophyseal pocket in association with the median olfactory pit;¹⁵ they communicate with the exterior by a common opening. In the 4.75-mm. embryo (fig. 7) the naso-hypophyseal invagination has shifted toward the dorsal side of the head, and the hood which has undergone extraordinary growth now forms the roof of the stomodaeum; at the bottom of the stomodaeal cavity the ectoderm has come in contact with the entoderm to form the pharyngeal membrane. In the 6.8-mm. stage the hood or upper lip has become straightened, and posterior thereto, on the dorsal surface, is located the opening of the naso-pituitary invagination. The dorsal surface of the hood is appreciably thickened, but the epithelium of its inner surface is relatively thin. Villi have appeared within the stomodaeum arranged in annular manner in front of the velum. The latter is a derivative of the pharyngeal membrane which remains into late stages of *Petromyzon* and marks the boundary between stomodaeal and pharyngeal cavities. These relations, as seen in an older specimen (27.6 mm.), are shown in figure 8.

In the larva of 42 mm. (fig. 9) the villi, apparently involved in the general anterior migration, have moved forward, and,

¹⁵ Compare Dohrn, loc. cit.; Ahlborn, *Zeitschr. f. wiss. Zool.*, 1883, Bd. 39, S. 191-294; Kaensche, *Zool. Beitr.*, Breslau, 1890, Bd. 2, S. 219-250.

in the young adult (fig. 10), are marginal in position. The upper lip in its later stages, as shown by Rathke's dissections, consists "chiefly of muscle fibers . . . by which the lip after expansion becomes again contracted." The lower lip, which is mostly connective tissue, contains a thin layer of muscle, and may be "somewhat contracted."¹⁶ Rathke regarded the villi as organs of taste.

In the young adult (fig. 58) the villi are macroscopic, very vascular, and covered with an epithelium containing an abundance of mucous cells. These cells have a very thick (4μ) and markedly striate cuticula, which, resisting compression in empty cells, may form a hemispherical cap over a downwardly tapering body. Thin dark cells with flat tops are frequently seen. But the well-preserved formalin specimen studied does not show that the cuticula is interrupted in places by sensory cells, each with five to ten stiff hair-like processes, in the manner described by Langerhans.¹⁷ In alcohol-fixed material Kaensche also was unable to find them, but he notes that Langerhans used fresh specimens. Thus the existence of sensory cells, so definitely described by Langerhans, awaits confirmation.

After the development of the villi, and altogether independently of them (Kaensche), the horny 'teeth' first appear, like the lips on the wrong side of the hypophysis. Their position within the funnel-shaped mouth, posterior to the villous cirri, is shown in figure 10. They are supported by a bar of cartilage (the annular cartilage) which, in sections, is a large object, rising into the pulp of the tooth (fig. 57). This pulp is a papilla of corium, capped by a thick and modified epithelium, the broad germinative layer of which is followed by a clear cornified zone. In the latter nuclei are absent, but intercellular spaces are retained. The cornified layer represents the crown of a developing tooth. External to it (fig. 57)

¹⁶ Rathke, H. *Anatomie des Querdors. Beiträge, Abth. 4*, Halle, 1827, S. 75-77.

¹⁷ Langerhans, P. *Untersuchungen über Petromyzon planeri*. Freiburg, 1873, S. 19-20.

there is a sheet of non-cornified cells, accumulating in a thicker mass toward the apex of the functioning tooth. These cells Kaensche named 'stellate,' since he observed their spiny intercellular processes as a conspicuous feature. It is an inappropriate term for the flat cells in my specimen. An outer zone of cornified cells, quite like the inner, completes the picture. Since the process of cornification is an interrupted one, provision is made for shedding the teeth, which are merely these cornified caps, one within another. In other vertebrates, the teeth are calcified cores of dentine, with a cover of enamel, which breaks through the epithelium to appear on the surface. Such a tooth of a dogfish embryo is shown for comparison in figure 59.

Henn (1923) finds that the teeth of cyclostomes are without genetical relations to the teeth of other groups.¹⁸ The small size of the dermal papilla, the absence of dentine, and the epidermal character of the tooth as a whole in *Petromyzon* support such a view. Yet, as Beard has pointed out, in *Myxine* and *Bdellostoma* the dermal papilla produces an imperfectly calcified tooth beneath the epidermal cornification.¹⁹ Warren ('02) and Bridge ('10)²⁰ are of the opinion that epidermal teeth represent a primitive stage in the evolution of teeth and dermal spines, to be followed by a later stage in which calcification supersedes cornification as a method of hardening. Until odontologists can agree whether *Petromyzon* has teeth, a decision as to the presence of lips may well be reserved. The mouth is certainly bounded by a muscular fold provided with vascular tentacles; and in describing the villi on the lips of human infants, West, in an important paper, has recently stated, "One is struck by the resemblance which they bear to the condition found in the mouth of *Petromyzon*."²¹

¹⁸ In Dean's Bibliography of Fishes, vol. 3.

¹⁹ Anat. Anz., 1888, Bd. 3, S. 169-172; Zool. Jahrb., 1889, S. 727-752.

²⁰ Warren, E., Quart. Journ. Micr. Sci., N. S., vol. 45, pp. 631-636; Bridge, T. W., Cambridge Nat. Hist., vol. 7, p. 248.

²¹ Carnegie Pub. no. 361 (Contr. to Embryology, vol. 16), 1925, pp. 23-45.

ELASMOBRANCHS

The mouth of elasmobranchs, commonly ventral, is large and crescentic. In some sharks, for example, in *Prionace glauca*, *Rhinobatus lentiginosus*, and *Squalus acanthias* (fig. 61) it has no connection with the nasal pits; but in the torpedoes and rays generally there is a pair of oronasal or nasobuccal grooves (fig. 63). The mouth opens into a spacious oral cavity. On its floor the mucous membrane is raised by the basihyal cartilage into a fold, more or less pronounced, forming the imperfect tongue,—“a protrusible tongue is never developed in fishes.”²² Small folds, generally corresponding in direction with the curve of the mouth, are present in front of the tongue. Cells secreting mucus are found, but there is absence “of all glands which are characteristically present in higher forms.”²³ Owen refers to the lips of most sharks and rays as “partially supported by labial cartilages”; Daniel recognizes the “membranous folds or lips” which bound the mouth; Gregory sees “all the elements of the face of man” and specifically includes lips.²⁴ A more adequate description follows.

Dogfish

Over the maxilla of *Squalus acanthias* (fig. 61) a prominent fleshy fold projects downward, almost concealing the teeth of the upper jaw. It is limited within by a pronounced groove, which in large specimens may attain a depth of 4 or 5 mm. The fold and groove (fig. 62) resemble to a striking degree the lip and labial groove of the mammalian mouth, and the cavity of the groove is properly a vestibulum oris. But toward the angle of the mouth the lip bifurcates, its two arms enclosing a depression into which fits an arm of the lower lip containing a labial cartilage. The upper arm of the upper lip likewise contains a cartilage, and as the cartilages of the two lips come together at the angle of the mouth they resemble, on a

²² Bridge, *Cambridge Nat. Hist.*, vol. 7, 1904, p. 252.

²³ Daniel, *Elasmobranch fishes*, Berkeley, 1922.

²⁴ Gregory, *Amer. Mus. Journ.*, 1917, vol. 17, p. 379.

small scale, the cartilages of the jaws themselves. Accepting Gegenbaur's interpretation of the labial cartilages as the upper and lower segments, respectively, of a premandibular branchial arch, Sewertzoff interprets the pocket behind this arch (seen between the diverging arms of the upper lip in fig. 62) as a rudimentary gill cleft which does not break through to the outer surface.²⁵ He goes even further and finds in the embryo of *Acanthias* a more anterior branchial outpocketing, which should indent the upper arm of the upper lip in figure 62. The chief labial cartilage of the upper lip lies behind this slight pocket; a smaller labial cartilage is in front of it and, as a whole, nearer the median line. Thus each half of the upper lip contains two cartilages, instead of one, as in the lower lip. For us it is a recondite problem whether these skeletal elements of the lips of sharks are regressive structures, as viewed by Gegenbaur and Sewertzoff, or are new acquisitions in process of further development as indicated by conditions in the higher vertebrates.

The relations of the upper lip of the dogfish, as seen in section, are shown in figure 14. Toward the median line it contains no cartilage. Its interior is filled with loose connective tissue, having many elastic fibers, sharply differentiated with Weigert's resorcin-fuchsin. It is not very vascular, but contains several nerve trunks, presumably sensory. Beneath the thick epidermis of the outer surface there is a dense tendon-like corium consisting chiefly of very coarse fibers parallel with the surface, crossed by perpendiculars at fairly regular intervals. The corium is bounded internally by a layer of fine elastic elements. Externally, an elastic basement membrane stretches under the epithelium, and on encountering the dermal spines above their expanded basal plates, it ensheathes them closely, and can be followed as far as their enamel. These spines, however, as seen in figure 14, are beyond the free portion of the lip. The epithelium is specially thickened toward the free edge of the lip and contains a few

²⁵ Sewertzoff, A. N. *Die Morphologie des Visceralapparates der Elasmobranchier*. *Anat. Anz.*, 1923, Bd. 56, pp. 389-410.

scattered slender papillae, sometimes distinctly vascular. On the inner surface of the lip the epithelium is thinner and the corium looser. This tough and elastic lip contains no muscle. The 'levator labii superioris' arises from the cranium, passes along the upper labial cartilage without being attached to it, and is inserted into the mandibular fascia; its function is not to raise the upper lip, but to protract the jaws.²⁶ None of the other muscles attached to the jaws have been found to enter the lips.

Internal to the upper lip there is a ridge of tissue covering the free margin of the palatoquadrate cartilage (which is the skeletal element of the upper jaw in selachians). This ridge bears the teeth, which develop along its inner surface, pass over its crest, and are then shed along its outer surface, their sites being marked by papillae of infiltrated or scar tissue. The denticerous ridge is the gingiva of figure 14.

Internal to the gingival ridge there is a transverse fold of soft tissue which is described in Cuvier's *Anatomie comparée* of 1835 as an internal lip (*lèvre intérieure*).²⁷ We had named this structure the palatine fold or valve (fig. 14, *v. pal.*) before reading the opinion of Allis that, under certain conditions, the corresponding structure in *Chlamydoselachus* "would strikingly suggest if it does not actually foretell, the secondary palate of mammals."²⁸ It had been evident that if the nasal pits should acquire internal openings above it, separated by a median septum which should grow down to meet it, mammalian conditions would result. But we had abandoned this idea as too speculative, before finding it expressed on the high authority of Allis. A corresponding infrapalatine fold or mandibular valve, though much less developed, rises toward it from the floor of the mouth.

The lower lip of the dogfish is similar to the upper, though much less developed (fig. 14). It is limited to the lateral

²⁶ Haller. *Entwicklung, Bau und Mechanik des Kieferapparates des Dornhais. Zeitschr. f. mikr-anat. Forschung*, 1926, Bd. 5, S. 783.

²⁷ Tome 4, Partie 1, p. 399.

²⁸ Allis, E. P. Homologies of the palato-quadrate of selachians. *Anat. Anz.*, 1913, Bd. 45, S. 355.

two-thirds of each half of the jaw. Medially, its absence suggests a greatly broadened frenulum.²⁹ The labial groove attains its maximum depth (4 mm.) near the angles of the mouth, and there the mouth ends in a pair of oblique clefts which, if prolonged, might reach the nasal pits, as in the skate.

The embryological development of the lips in *Squalus* may be studied in the sagittal series of the Harvard Collection, which are among the specimens used by Scammon for his *Normentafeln* in 1911.³⁰ At 5 mm. ectoderm and entoderm have fused to form the oral plate. At 22 mm. (fig. 11) the plate has gone, except for a remnant, just anterior to which the hypophyseal diverticulum is still attached to the roof of the mouth. The oral roof and the floor of the forebrain are no longer in complete apposition. Mesenchyma has begun to separate these two epithelial surfaces, and the roof of the mouth shows certain obscure transverse folds. In the 50-mm. embryo (fig. 12) the significance of these folds is evident. There are then three of them, of which the most anterior, limited behind by a groove, will form the lip of the adult; the middle one is the gingival ridge; the most posterior, situated just behind the dental lamina, is the palatine valve. A later stage is shown in figure 13, from an embryo of 159 mm. The balanced relation between the palatoquadrate cartilage and Meckel's cartilage, each with a labial lamina or groove in front of it and a dental lamina or groove behind it, is strikingly apparent. Usually the elasmobranch lips are set off by a groove. This is true of the upper lip, and of the lower lip laterally; but more medially the latter presents a solid lamina (fig. 13), which is the common condition in embryonic mammals.

Raja and Torpedo

In *Raja*, but not in *Squalus*, the oblique clefts at the angles of the mouth extend forward to the nasal pits, thus forming

²⁹ According to Jordan and Evermann, *Chimaera* has "lips thickish, the lower with a frenum."

³⁰ Scammon: *Squalus acanthias*, Heft 112 in *Keibel's Normentafeln zur Entw. d. Wirbeltiere*, 1911.

the oronasal grooves. They extend also posteriorly; and curving toward each other beneath the mandibular rami, they cross the midline as a series of inframandibular sulci (fig. 63). Human embryos of the 8 to 10-mm. stage, and larger, show a corresponding furrow beneath the lower jaw, which accounts for their characteristic 'double chin.'³¹ But the dogfish (fig. 61) has nothing of the sort. In the skate, between the upper lip and the teeth, there are variable loose folds, quite conspicuous on the right of figure 63. They seem to be merely the slack when the jaw is retracted. The upper lip is much better developed than the lower, and in the section of a 48-mm. embryo (fig. 18) with a distinct labium superius, no trace of a lower lip is indicated.

The embryological origin of the lip in torpedoes is shown in figures 15 to 17. These sections are quite like those of *Squalus*, except that the dental laminae open into sulci toward the surface, and the inframandibular furrows have appeared in figure 17. The cartilage shown in the upper lip is a median structure not found in the dogfish. Such examples of lips as have been presented presumably represent the typical selachian condition.

Selachian lips in relation to denticles and villi

Williamson's paper in '49, "On the microscopic structure of scales and dermal teeth," and the brilliant studies of Owen and Hertwig which followed, have established as a most familiar fact that selachian teeth and placoid scales are "identical in essential structure as well as in their manner of development."³² But we cannot agree with Bridge that in the embryo dermal spines and teeth form a continuous series and "it is only later, when lips become apparent, that the con-

³¹ Compare His as to the 'Doppelkinn'—Die Entwicklung der menschlichen und thierischer Physiognomien.—Eine Skizze. Arch. f. Anat. u. Entw., 1892, S. 394.

³² Williamson, W. C. Phil. Trans. Roy. Soc. London, 1849, pp. 435-475; Owen, R. Odontography, London, 1840-1845; Hertwig, O. Bau u. Entw. d. Placoidschuppen u. d. Zähne, Jena. Zeitschr., 1874, Bd. 8, S. 331-404; Hautskelet d. Fische, Morph. Jahrb., 1876, Bd. 2, S. 328-395.

tinuity of the teeth and dermal spines is interrupted and the two structures assume their distinctive characters.”³³ That conclusion he supports with Gegenbaur’s figure of the lower jaw of a young *Scyllium*, showing the teeth with their spines pointing inward, and scales of the adjacent skin with spines pointing outward, between which there are scales with spines pointing both ways, making a perfect transition. We have found Gegenbaur’s figure decidedly misleading, as also his statement that “the presence of these structures [the teeth] in the primitive buccal cavity is explicable from the fact that it was formed by an invagination from the exterior.”³⁴

There is apparently no selachian mouth which has a general lining of dermal denticles, certain of which become hypertrophied to form the teeth. On the contrary, the teeth appear as primary structures, decidedly segregated in early embryonic stages. They arise as specialized papillae of the corium along the labial wall of the dental lamina; the lingual wall of the lamina, lacking the power to form teeth, serves as a covering—the ‘thecal lamina’ of Bolk. Subsequently, teeth form in other situations. The dermal denticles of the lower jaw of the dogfish are directed distally, so as not to oppose the forward movement in swimming (fig. 14, with an enlarged one at *b*) and the nearest teeth point into the mouth, to retain food (fig. 14, *a*). Very likely in other forms, as figured by Gegenbaur, the transition between teeth and scale is bridged by T-shaped structures of intermediate size, which point both ways. Thus the teeth on either half of the lower jaw of *Hep-tanchus*, as figured by McDonald and Barron,³⁵ are serrated predominantly to the right and left, respectively, with a median tooth serrated both ways. But this is no evidence that the right teeth were derived from the left or vice versa. The fact which Gegenbaur well knew, that teeth may develop in entodermal territory, is in itself sufficient to show that they

³³ Cambridge Nat. Hist., vol. 7, pp. 248–249.

³⁴ Gegenbaur, C. Elements of comparative anatomy. London, 1878, p. 550. The figure is in *Vergl. Anat. d. Wirbelthiere*, Bd. 2, Leipzig, 1901, S. 40.

³⁵ See Daniel, p. 124.

are not merely scales impocketed into the mouth and there hypertrophied.

This rather fine distinction is of special importance in our particular problem. No denticles are found on the inner surface of the selachian lip, i.e., in the region of transition between scales and teeth, where in the cat the tooth-shaped villi are located. The latter, therefore, cannot be interpreted as selachian denticles. Furthermore, the labial villi of mammals are non-calcified structures rising above the general level of the mucous membrane. Although Röse insists (against Hertwig) that "die erste Anlage der Zähne zeigt sich bei *Crocodylus biporcatus* in Form von freien, über die Oberfläche der Mundschleimhaut hervorragenden Papillen,"³⁶ it is certainly characteristic of selachian teeth and spines that they do not project until calcified.

Toward the upper lip of the young dogfish (fig. 14) the dermal spines end quite abruptly. An occasional broad and characteristic papilla on the outer surface of the lip, causing no elevation, is evidently a denticle in process of development, or perhaps in arrested development. But the epithelium toward the labial margin becomes very thick. With the scattered slender vascular papillae which enter it, though causing no elevations, it is quite suggestive of the pars villosa of the human lip. The relation, if any, between the slender papillae of the dogfish lip and the broad ones which are abortive denticles, cannot be easily determined: they appear to be altogether independent formations.

TELEOSTS

The extraordinary development of the lips of teleosts makes them, of all classes, the most interesting for the present study. That was recognized by Allis, whose account of "the lips and the nasal apertures in gnathostome fishes" constitutes the essential literature of this subject.³⁷ The primary, secondary,

³⁶ Röse, C. Ueber die Zahnentwicklung der Reptilien. Deutsche Monatsschrift f. Zahnheilkunde, 1892, Jahrg. 10, p. 129.

³⁷ Allis, E. P. Jour. Morph., 1919, vol. 32, pp. 145-205. (Paper dated 1916. Through an oversight, the plates, though accompanied by a list of abbreviations, are without lettering and consequently sometimes difficult to interpret.)

and tertiary lips which he described in the teleosts, and found represented in selachians and dipnoans, will here be presented in a simpler manner, and quite differently, since it is the result of a wholly independent investigation; yet the main conclusions of Allis are strikingly verified. We have selected three common and widely distributed fishes—the cod, carp,

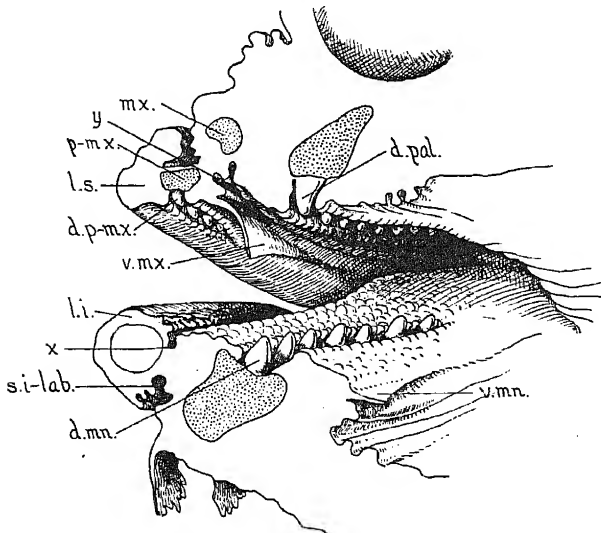


Fig. 1 Toadfish (*Opsanus tau*). Sagittal section of the head. $\times 3$. *d.pal.*, *d.p-mx.*, *d.mn.*, palatine, premaxillary, and mandibular teeth, respectively; *li.*, lower lip; *l.s.*, upper lip; *mx.*, maxilla; *p-mx.*, premaxilla; *s.i-lab.*, infralabial sulcus; *v.mn.*, mandibular valve; *v.mx.*, maxillary valve; *x* and *y*, morphologically equivalent sulci.

and trout—as presumably typical of the vast array. The toadfish has been added, since embryological sections of that species were already at hand.

Toadfish (Opsanus tau)

In a parasagittal section of an adult, passing along the medial surface of the orbit (fig. 1), the single row of strong, blunt palatine teeth (*d.pal.*) in the upper jaw and the corresponding row of mandibular teeth in the lower jaw (*d.mn.*) serve as the essential landmarks for comparison with the

selachians. The palatine valve of the shark, behind the palatine teeth, has disappeared, but the corresponding structure of the lower jaw—the mandibular valve (*v.mn.*)—is well developed. Just behind the palatine teeth, and also in front of them, there is a row of soft nodular elevations of the mucous membrane. Several rows of similar nodules are seen on either side of the mandibular teeth.

Replacing the simple curtain-like upper lip of the dogfish, there is here a protractile upturned structure, containing two bones instead of two cartilages, and provided with a row of premaxillary teeth (*d.p.mx.*) which does not extend very far laterally. These teeth, like the others, are bordered on either side by a line of vascular nodular elevations of the mucosa. Between the teeth and the anterior beaded fringe there is a groove of some depth, forecasting a new labial sulcus; and in front of the beaded fringe a shallow depression may be considered as separating an internal *pars villosa* from an external *pars glabra*. However, this new secondary upper lip is still very ill-defined. Behind the anterior row of teeth there is a new palate-like fold, called the maxillary valve (*v.mx.*), which functionally replaces the palatine valve of selachians.

Cuvier recognized that back of the anterior teeth, and almost always in both jaws, “there is a sort of membranous velum or valve . . . the effect of which is to prevent food, and especially the water taken in for respiration, from escaping through the mouth.” He found this valve well marked in the genus *Zeus*, and noted that it exists ‘dans une infinité d’autres poissons’ (*Hist. nat. des poissons*, Paris, 1828, T. 1, p. 497). Dahlgren, watching living fishes in aquaria, saw the valves in action in over fifty species—‘no teleost has been found without them’—and unaware of Cuvier’s reference, he wrote his excellent account of ‘the maxillary and mandibular breathing valves of teleost fishes’ (*Zool. Bull.*, 1898, vol. 2, pp. 117–124). Cuvier or perhaps his editor, Duvernoy, made no distinction between the palatine valve of selachians and the maxillary valve of teleosts, both being included under ‘lèvres intérieures’; but their radical topographical difference has been noted by Allis and others.

The lower lip of the toadfish is by no means a replica of the upper, though embryologically it arises in similar fashion

(fig. 20, from an 8-mm. specimen). Medially, on either side, a premandibular cartilage develops within it (shown in section in fig. 1), but no bone, and it has no teeth. No breathing-valve is associated with it. Its crest shows medially some obscure nodules, apparently comparable with those of the upper lip which are in relation with the premaxillary teeth. The entire structure is rather loosely attached to the lower jaw, since a deep infralabial sulcus (*s.i-lab.*) rises from below toward a groove, *x*, which separates the premandibular formation from the nodulous gingival zone. Morphologically corresponding to *x* is the groove *y* in the upper jaw. This arrangement suggests that the premandibular lip will be suppressed in higher vertebrates or will merge in a secondary lower lip, whereas the primary upper lip will become an integral part of the upper jaw with or without the formation of a secondary upper lip.

A curious condition, in the toadfish, results from having one set of teeth in the lower jaw and two sets in the upper. In general, in the animal series, the primitive occlusion of the mandibular teeth with the palatine gives place to occlusion of the mandibular teeth with the maxillary and premaxillary, accomplished by a forward growth of the lower jaw. Subsequently the palatine teeth disappear. The toadfish is in a transition stage. Laterally, its mandibular teeth bite against the palatine. Medially, they bite against the central premaxillaries, but the more lateral premaxillaries bite against the lower lip. This extraordinary maladjustment of upper and lower teeth is shown in figure 21, a section of a 42-mm. specimen. Comparison with figure 20 shows how different is the course of development taken by the two primary lips and summarizes what we have said of this fish. In the cod, what seems a far more satisfactory occlusion has been effected.³⁸

³⁸I am indebted to Dr. Lewis for formulating the comparison between the fish and amphibian lips as presented throughout this and the following sections. He has made the finished drawings of figures 19 to 21, 25 and 26, 28 to 32, and some others; figures 1 to 3 and 23 were redrawn by Mr. Aitken under his direction, during my stay in Chicago.

Cod (Gadus morrhua)

The several folds about the mouth have been sketched in figure 23. The entire margin of the upper jaw is supported by the premaxillary bone. Internal to the premaxillary at the angle of the mouth, and extending forward above the premaxillary under the eye, is a fold formed about the maxillary bone. It passes out of the region of the lip as seen in figure 2 (*mx.*, compare fig. 1).

As stated by Kesteven,³⁹ the maxillae and premaxillae of the majority of teleostean fish constitute an adventitious jaw. That they are "homologous with the labial cartilages of the elasmobranch fish is based on the relation of both structures to the forepart of the skull and to the lips." With that portion of his contention we can agree, but not with his 'somewhat startling ideas' regarding their homologies in the higher vertebrates. The older view seems unassailable, namely that the premaxillary bone loses its lateral part and becomes intermaxillary, allowing the maxilla to form all the lateral portion of the jaw in higher vertebrates.

Above the maxillary fold in the cod there is a supramaxillary fold, which in part of its course contains a bone variously called lacrimal, peri-orbital, infra-orbital, etc., with the homologies of which happily we are not concerned. This fold represents the tertiary lip of Allis, which, as a lip, is 'found only in the Dipneusti' (*l.c.*, p. 184). In mammalian embryos its homologue is a portion of the maxillary process bounding the lacrimal groove laterally (Allis, p. 193). Thus it extends downward from the region of the inner canthus of the eye to the middle of the cheek, and in none of the animals which we have studied is there valid reason to consider it a lip.

The lower jaw is formed by a broad mandibular element, which is seen both above and below the premandibular fold lying in front of it. This premandibular fold tapers laterally, contains a cartilage, and constitutes the primary lower lip. That it is comparable with the primary upper lip has been

³⁹ Kesteven, H. L. A new interpretation of the bones in the palate and upper jaw of fishes. *Journ. Anat.*, 1922, vol. 56, p. 315.

shown in the toadfish. But whereas the secondary upper lip is only a small part of the primary upper lip, the secondary lower lip includes all of the premandibular fold together with a portion of the mandibular fold. In fishes in which the premandibular fold is not developed, the lower lip is altogether a derivative of the mandibular fold.

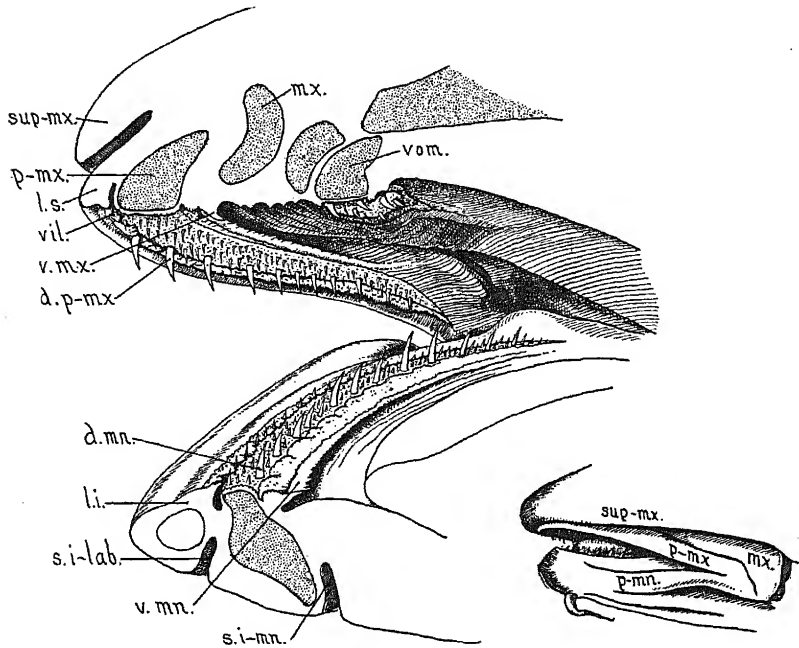


Fig. 2 Codfish (*Gadus morrhua*). Sagittal section of the head. Natural size. Lettering as in figure 1, with the following additions: *p-mn.*, premandibular fold; *s.i-mn.*, inframandibular sulcus; *sup-mx.*, supramaxillary fold; *vil.*, labial villi; *vom.*, vomer.

The paramedian section of the cod's head (fig. 2) is readily comparable with that of the toadfish. In the cod the row of palatine teeth is lacking, but the median extension of that row on the vomer is retained; a few vomerine teeth appear in the section, with wrinkled and somewhat nodular folds of mucous membrane on either side, suggestive of conditions in the toadfish. There are several ranks of premaxillary teeth, occupy-

ing a crescentic dentigerous area widening to 11 mm. medially, but ending abruptly before reaching the midline. The mucous membrane of the roof of the mouth can thus pass uninterruptedly to the frenular portion of the upper lip. Internal to the upper teeth there is a row of nodules along the attached border of the somewhat rudimentary maxillary breathing-valve. Externally there is a well-defined labial sulcus, in places 3 mm. deep. The lip, without magnification, shows a fringed inner part or villosa, and smooth outer glabra. It is overhung by the supramaxillary fold.

The lower jaw has several rows of mandibular teeth, which now occlude effectively with the premaxillaries. Within the mouth these teeth are bordered by nodular excrescences of mucous membrane which, as in the upper jaw, are along the attached border of the rudimentary breathing-valve. The extensive lower lip contains the premandibular cartilage in its medial part, and shows a fringed crest and smooth outer declivity. Since the villous fringe follows the line of the teeth, it is only medially that it can be regarded as crowning the premandibular fold (fig. 2). There is a deep infralabial sulcus as in the toadfish, and a still deeper inframandibular sulcus. A tongue, much more like that of higher animals than is found in most fishes, rises from the floor of the mouth.

A sagittal section of the lips of a full-grown cod, not far from the median line, is shown in figure 22. The skin of the pars glabra is thinner, softer, and less deeply pigmented than ordinary skin. It is crossed by shallow furrows extending out from the spaces between the villi. A better idea of the villi is obtained from the photograph, figure 64. The largest of them, 3 mm. tall, are found on the upper lip near the median line. These are compound structures, consisting of a central mound with ten to twenty rounded marginal lobulations. Laterally, they become smaller, lose their secondary outgrowths, and are reduced to simple nodules 0.5 to 1.5 mm. high. Even these disappear toward the corners of the mouth, where the crest of the lip loses all its serrations and zones, and becomes a simple smooth ridge or fold. The

villi in a single row along the lower lip are not so broad as those of the upper lip, and in many instances are not compound. They, too, become smaller and disappear toward the corners of the mouth. These excrescences or villi occur not only pressed against the teeth, but in the form of small button-like nodules, similar to the secondary outgrowths of the large labial villi, they may be found throughout the length and breadth of the dentigerous zones. Among the teeth they are irregularly distributed. They occur also on the frenula of upper and lower lips. Internal to the plates of teeth, they form another continuous fringe. This distribution may be compared with that of the human villi, found both on the lips and on the gums.

Each villus consists of a very vascular connective-tissue core, covered with thick stratified epithelium. The connective tissue forms a single primary papilla in the simple villi, which, in compound forms, sends a digitation into each of the peripheral nodules. Within the larger villi secondary papillae may be found at the top and adjacent portion of the sides of the primary papillae. The epithelium is thinnest over the tops of the secondary papillae. It heaps up over the lateral surfaces, where it is commonly from fifteen to twenty cells deep. The outer cells are not greatly flattened. Scattered about, but most abundant at the base of the depressions between the mounds, there are cells distended with mucus. Of greater interest is the profusion of taste buds, which, though found elsewhere, are particularly abundant in the labial villi. These buds occur on the free surface of the villi, at the top (fig. 64), and not on the sides as, for example, in the foliate lingual papillae of the rabbit. Conspicuous nerve trunks entering the villi are further evidence of their highly sensory function.

Jonathan Couch (Trans. Linn. Soc., London, 1825, vol. 14, p. 72) reported that "filaments between the teeth and lips of the cod seem designed to enable it to discover and select its prey. And how well they are able to fulfill their object appears from the instance of a codfish" [etc., referring to a large one in good condition, which seemed congenitally blind].

Leydig (Zeitschr. f. wiss. Zool., 1851, Bd. 3, and again in his Lehrbuch, 1857, p. 197) figured a very round taste bud on the top of a vascular papilla from the lip of *Leuciscus dobula*, a cyprinoid roach. F. E. Schulze (Ueber die becherförmigen Organe der Fische, Zeitschr. f. wiss. Zool., 1863, Bd. 12, S. 218-222) assigned to them a gustatory function, which Herrick has conclusively established (Jour. Comp. Neur., 1901, vol. 12, pp. 329-334; Bull. U. S. Fish Com., 1903, vol. 22, pp. 237-272). Bateson (Journ. Marine Biol. Assoc., London, 1890, N. S., vol. 1, p. 225-256) records their presence on the lips of four species of *Gadus*, but does not associate them with labial villi. I find them present in the epithelium of the dentigerous zone, especially on the low villi found there, and also on the nodular formations internal to the dental arch, but nowhere in the codfish are they more abundant than on the labial villi. None were observed on the villi of the toadfish.

Carp (Cyprinus carpio)

This fish has thick pouting lips, the limits of which are ill-defined, since no teeth develop in either jaw (fig. 3). "The margin of the upper jaw is formed by the premaxillaries alone," and beyond this bone the lip projects forward. Suspended above it there is a fold suggestive of the supramaxillary fold in the cod, but since it contains the maxillary bone, we consider it the maxillary fold. If that is correct, then the supramaxillary fold of the carp is limited to the sides of the head. Comparison of figure 3 with the section of the toadfish (fig. 1) corroborates this opinion.

The position where the premaxillary teeth should erupt is indicated in the carp by the tract of nodules beneath the premaxillary bone. In front of them is a distinct labial sulcus, and then the lip, feebly furrowed anteroposteriorly. In other cyprinoids the lip may be more deeply plicate (as in *Catostomus duquesnii*), or tuberculate, i.e., with low nodules or 'villi,' as in *C. occidentalis*. In the latter its margin is upturned, as it is in the toadfish. In all cases the furrowed or nodular portion of the lip bears taste buds, which are abundant in the section of *C. duquesnii* photographed as figure 65.

The carp has a large maxillary breathing-valve (fig. 3), behind which is a palatine prominence—"thick, soft, minutely

granular, and very sensitive popularly named 'carp tongue.'⁴⁰

The floor of the mouth displays no striking features; a tongue, such as is found in the cod, is wanting. There is no premandibular cartilage. The infralabial sulcus, which does not cross the midline, is well marked laterally. The lip thus

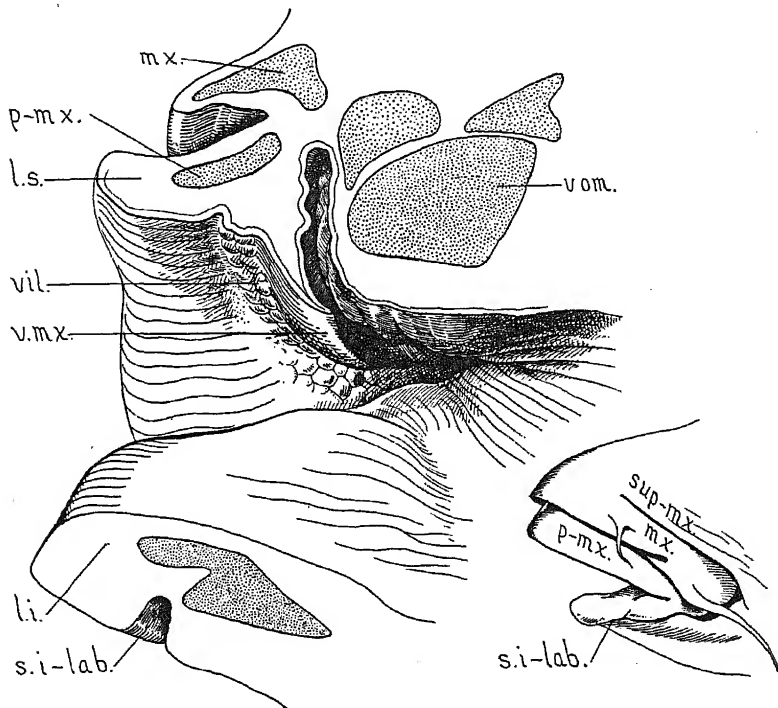


Fig. 3 Carp (*Cyprinus carpio*). Sagittal section of the head. $\times 3$. Lettering as in figures 1 and 2.

becomes a thick, rounded, and somewhat everted margin of the jaw. In *Catastomus duquesnii* the lower lip is further everted, growing back under the ramus of the jaw on either side as a rounded lobe; such bilobate lips are described as 'deeply incised.' Like the upper lip, the lower, in certain

⁴⁰ Yarrell, *British Fishes*, 3rd ed., London, 1859, vol. 1, p. 356.

species, may be plicate, and the crests of the anteroposterior plicae may be crenate, so as to appear fringed.

Brook trout (Salvelinus fontinalis)

As compared with the section of the toadfish (fig. 21), that of the trout is simple and compact (fig. 19), in many points resembling the amphibians. The premaxillaries are no longer protractile. The maxillaries have descended to form the lateral margins of the upper jaw, and bear teeth which are lateral extensions of the premaxillary series. The primary upper lip, in which the premaxillary bone developed, has become thoroughly incorporated in the front of the head and there is no secondary upper lip. Maxillary valve and palatine teeth make clear the orientation in relation with figure 21. The lower jaw of the trout dispenses with the premandibular lip and cartilage—large structures in toadfish and cod, which are in process of elimination in the carp—and in this it is strikingly amphibian. The mandibular valve is shown in the section, and lingual teeth make the tongue conspicuous. Since the skin of the front of the head turns in smoothly at the mouth and arrives at the dental area in both jaws with no indication of a labial groove, the trout is a fish with no trace of lips.

AMPHIBIANS

Urodeles

In the embryo of *Amblystoma* the mouth is at first ventral, and at 8 mm. (fig. 24) is closed by a plug of cells extending to the hypophyseal outgrowth (some of the cells in this plug contain yolk granules, as noted by Eycleshymer in *Necturus*). Subsequently, the mouth becomes terminal, and sagittal sections of the head at 26 mm. are very much like those of the trout at 25 mm. (compare figs. 19 and 25). In neither animal has the mandible advanced far enough to bring the mandibular teeth into effective occlusion with the premaxillary; in both, the vomeropalatine teeth are well back in the roof of the mouth. But *Amblystoma* lacks the breathing-valves; and

laterally the lower lip, feebly marked out in figure 25, becomes an overhanging structure with a deep infralabial sulcus. This is shown in figure 32*A*, from a 12-mm. specimen. Still more laterally (fig. 32*B*) the labial margin of the upper jaw descends below the lower lip. It then enters the infralabial sulcus to join the lower lip and form the corner of the mouth. The resulting condition is readily comparable with that in the dogfish (fig. 62) if we consider that in *Amblystoma* the inner ramus of the upper lip has been suppressed.

Although such corners of the mouth can be observed in adult *Amblystoma*, they are more strikingly present in adult *Necturus*, in which Cope described the lips as follows:

The upper lip is rather full and has a thin edge. It overhangs the lower lip, concealing the posterior [i.e., posterolateral] part of it, and embracing it at the canthus, since it is attached within the groove which bounds it below. The lower lip is decurved, and the anterior part is deeper, or more convex downwards, than the posterior half, and is separated from the corresponding part of the opposite side by a considerable interspace which is without groove.⁴¹

A section of *Necturus* (31 mm.), for comparison with *Amblystoma*, is shown in figure 26. It presents a primitive feature in that the mandibular teeth occlude with the vomeropalatine. There is a thick lower lip with a clumsy labial sulcus, and tissue in front of the premaxillary teeth is ready for invasion by a corresponding sulcus, when it will form an upper lip.

In a large *Cryptobranchus* (35 cm.), though there is a shallow labial groove which follows the curve of the broad upper jaw to the ends of the dental area, shown at its deepest in figure 27, there is no corresponding groove on the lower jaw. There is no groove for either lip in the 60-mm. *Spelerpes* (fig. 28). This section shows the mandibular and premaxillary teeth in occlusion, with the vomeropalatine teeth far back in the mouth. Transverse maxillary and mandibular folds (*v.mx.* and *v.mn.*), suggestive of the breathing-valves

⁴¹ Cope, E. D. *Batrachia of North America*. Bull. U. S. Nat. Mus., 1889, no. 34, p. 24.

of fishes, appear in this section; but the mandibular fold rises from the floor of the mouth instead of extending backward from the mass of tissue investing the teeth; and the maxillary fold, though correctly placed, is hardly thin enough to be valvular. An interesting feature of *Spelerpes bilineatus*, as described by Cope,⁴² is the "slight obtuseness of the lip on each side of the muzzle to represent the cirrous appendage of the larva, which is sometimes persistent, thus presenting the characters of the supposed species of *S. cirrigera*." In

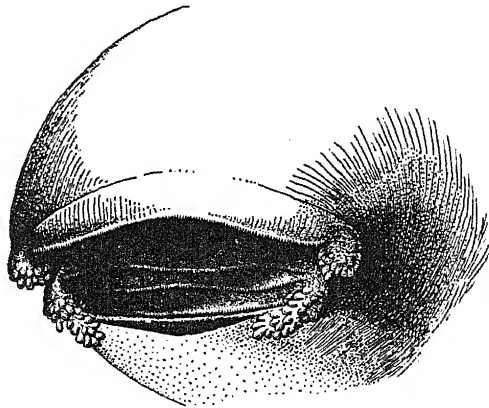


Fig. 4 Mouth of a 17-mm. toad-tadpole (*Bufo lentiginosus americanus*). $\times 20$.

the type specimen of *cirrigera* Cope describes the cirri of the upper lip as "cylindrical and a little knobbed at the ends, extending downward past the lower jaw: they are about 0.05 of an inch in length: the appearance presented is not unlike that of the muzzle of a walrus." We have had no opportunity to observe the larval cirri of *Spelerpes*.

Anurans

The tadpoles of frogs and toads, unlike those of urodeles, pass through a stage in which the mouth is at the bottom of a pocket (vestibulum oris, Mundbucht, vestibule buccal) formed by specialized projecting lips, each of which bears several rows of hooked cornified cells or 'teeth' (fig. 4). At

⁴² Loc. cit., p. 164.

the angles of the mouth there are conspicuous cirri or papillae. These familiar structures have been described many times, perhaps first by Swammerdam. In Floyd's translation, with reference presumably to the tadpole of *Rana fusca*, we read:

The aperture of the mouth consists of an under jaw and an upper one, both moveable, of an extreme blackness, and armed with very small teeth like a saw, with which, considering its strength and size, the little animal is able to bite exceeding hard. These parts seem to be made of a slender, horny, and pretty flexible bone. There are, moreover, both above and below the opening, a great many little horny bones of the same kind, furnished with a multitude of little black teeth. All these little bones are placed on some muscular and very white plaits which serve the animal like so many lips. The skin lying on each side beneath the mouth, consists of a great number of white papillae (Swammerdam. *Book of Nature, or History of Insects*. London, 1758, p. 116).

Excellent modern descriptions are those of Heron-Royer and van Bambeke, with the supplementary study by Gutzeit, and a brief but valuable report by Miss Hinckley.⁴³ At a stage when this apparatus is well developed (fig. 29A), a rostral cartilage (Dugès; *cartilago labialis inferior*, Gaupp⁴⁴) supports a continuous cornified beak, composed of V-shaped cellular laminae stacked one within another. Suspended below it is the lip, deeply undercut by an infralabial groove such as was seen laterally in *Amblystoma* (compare fig. 32). But in the tadpole the lip extends across the median line; and its everted inner surface—the outer surface as it hangs pendent—bears three transverse ridges of thick, stratified epithelium, within which vertical columns of cells become cornified, forming incurved horny hooks or 'teeth.' Gutzeit estimated that there were 385 of these teeth on the lower lip of *Rana fusca*. The base of each functioning tooth is invaded

⁴³ Heron-Royer et Ch. van Bambeke, *Arch. de Biol.*, 1899, T. 9, pp. 185-309; Gutzeit, *Zeitschr. f. Zool.*, 1890, Bd. 49, S. 43-70; Hinckley, M. H., *Proc. Boston Soc. Nat. Hist.*, 1882, vol. 21, pp. 307-315.

⁴⁴ Earlier literature is comprehensively treated by Gaupp, *Primordial-Cranium von Rana fusca*, 1893, *Morph. Arbeit*, 1892, Bd. 2, pp. 275-481. We cite also Dugès, *Sur l'ostéologie et la myologie des batraciens*, Paris, 1834, pp. 1-216.

by the curved top of one destined to replace it, and this in turn by another, so that they have been likened to 'piles of liberty caps.' In section they are seen in the photograph, figure 60, and in van Bambeke's lithographs, but it remains for some one to reconstruct the individual cells before this most remarkable transformation of stratified epithelium can be adequately visualized. An epithelial mound with its crest of horny teeth constitutes a 'pectinate ridge' (van Bambeke) or 'fringed fold' (Miss Hinckley).

The inferior rostral cartilages appear to arise in continuity with each other and with the anterior ends of Meckel's cartilage. Stöhr states explicitly, *Die ersten Skeletanlagen des Anurenkopfes sind: 1) Untere Lippenknorpel, MECKEL'scher Knorpel und Quadrata, die zusammen ein Continuum bilden; diese Anlage ist unpaar, jedoch verräth die Gruppierung der sie konstituierenden Zellen eine Zusammensetzung aus zwei Stücken.*⁴⁵ A median, more cellular disc separates the lateral halves of the rostral cartilage, so that it is usually described as paired. A more difficult question is its relation to Meckel's cartilage. Though there is cartilaginous fusion and continuity between the two, the rostral cartilage appears as an independent element, spliced to Meckel's cartilage, and overlapping it so that laterally, toward the corners of the mouth, a single sagittal section may show detached sections of both elements (fig. 30). It can hardly correspond with the premandibular cartilage of fishes, since the mandibular bone develops in front of it (fig. 31). Thus it is either a new element, or, as Stöhr believes, an adaptive modification of the terminal part of Meckel's cartilage.

Near the median line, on either side, and extending straight back from its attachment to the under surface of the rostral cartilage, there is a long slender muscle—the genio-hyoid of Dugès (figs. 60 and 30, *m.g-h.*). A much shorter and smaller muscle arises from the posterior side of Meckel's cartilage near its tip, passes toward the depth of the infralabial sulcus,

⁴⁵ Stöhr, Ph. Zur Entw. des Anurenschädels. Zeitschr. f. wiss. Zool., 1881, Bd. 36, S. 79.

and enters the lip close to its inferior epithelial surface (figs. 29*A* and 30, *m.l.*). It is inserted into the adjacent subepithelial tissue of the lip, with fibrous strands radiating toward the pectinate ridges. These muscles are very definite, but other strands described by Dugès—a 'rostro-labial' muscle—we have not been able to identify. The lips are predominantly fibrous and not muscular, and yet, as Dugès states, they "jouissent d'une mobilité notable, et même d'une certaine force." Swammerdam observed that they can be "opened, shut, and moved in various ways, seizing food and helping to convey it to the mouth." Miss Hinckley reported that the fringed folds "are capable of being laid back when the tadpole wishes to reject any substance held by the fringe."

The upper lip is external to a cornified beak which descends in front of the lower beak when the mouth is closed (fig. 29*A*). The upper beak is supported by a rostral cartilage or pair of cartilages (clearly modeled by Gaupp in *R. fusca*). According to Stöhr, Die oberen Lippenknorpel entstehen durch Abschnürung von den Balkenanlagen und dokumentieren sich hierdurch als vorderste Abschnitte der seitlichen Schädelbalken.⁴⁶ Gaupp suggests that their interpretation calls for further investigation of the upper labial cartilages 'bei den Fischen.' They seem to be a maxillary or intermaxillary cartilage—a new formation, destined to disappear. Anterior to the upper beak in the tadpole is the upper lip, less developed than the lower, and having only two pectinate ridges, the inner being deficient toward the median line. There is nothing to correspond with the infralabial sulcus. This lip in our sections is notably non-muscular.

At the corners of the mouth there are papillae (fig. 29*B*) which, judged by uninjected specimens, are quite vascular. Stricker, Schulze, and others, as reviewed by van Bambeke, have found within them transversely placed cells suggestive of tactile corpuscles. Nerves enter them (Gutzeit), but it is admitted that they require investigation with special methods to confirm their presumably tactile function. No

⁴⁶ Loc. cit., p. 86.

sensory buds were observed in their epithelium. Within the oral cavity there are occasional conspicuous plicae, or tall wide villi, of similar structure, which disappear during metamorphosis.

In the older tadpoles the horny teeth, and ultimately the horny beaks, are shed (fig. 30) and the lips atrophy. They have wholly gone in the 10-mm. toad (fig. 31), which shows the oral contour of the adult. Spicules of bone are developing in front of the rostral cartilage of the lower jaw, and the remnant of the cartilage in the upper jaw is completely surrounded by the intermaxillary bone. Lipless, toothless jaws are the attained result.

REPTILES

Crocodylia

In the unhatched crocodile, what appear to be hard and inflexible lips are readily found (fig. 5A, p. 375), but the teeth emerge through their crests, and the labial tissue, if present, is adherent to the jaws: "Maxillae sinuosae, labiis liberis destitutae."⁴⁷ Barge, in a special study of the lips of reptiles, goes further and finds in crocodiles "not a trace, not an indication of a lip."⁴⁸ (A further comment on the labial region of crocodiles will be made in the section on birds, with which they have features in common.)

Lacertilia

Young embryos of a common geckonid lizard of Jamaica, *Aristelliger praesignis*, are without lips at the stage when the dental laminae invade the mesenchyma of both jaws (fig. 33). The mandibular dental lamina grows down behind the bone developing around Meckel's cartilage. At 8.8 mm. (fig. 34) another lamina has begun to descend in front of the bone

⁴⁷ Hoffmann, in Bronn's *Thierreich*, Bd. 6, Abth. 3, S. 1058, referring to all 'Crocodylina.'

⁴⁸ Barge, J. A. J. *Zur Morphologie der Lippe. I. Über Lippenbildung bei den Reptilien.* *Zeitschr. f. Anat. u. Entw.*, 1927, Bd. 82, S. 694-719. (I am indebted to Dr. H. L. Weatherford for calling attention to this important paper, and for other valuable suggestions.)

of the mandible. This in its upper part is the labial lamina, but below it gives rise to a series of ramified glands and constitutes the 'glandular band' of Bolk,⁴⁹ or lamina glandularis.

Originally the excretory ducts to which the glandular lamina gives rise "open on the surface in all reptilian embryos," but later they may closely approach the alveoli of the teeth, with which they correspond in number. Bolk therefore criticises those who call them 'labial' glands, though he does not wish to stress the fact that "lips in the accepted sense are wanting in reptiles," and proposes to name them 'tooth-glands.' However, he recognizes that they may remain independent of the teeth, "in which case a groove is usually formed, bounded on the medial side by the os dentale, and laterally by a fold of soft tissue, on the bottom of which the ducts open: by the development of this protecting wall of soft tissue there comes about an anatomical condition not unlike that in mammals with real lips." It is also not unlike that in *Amblystoma* (fig. 25) or in *Squalus* (fig. 14), except that in them no glandular structures are found at the bottom of the groove. Bolk's pupil, Barge, who continued the investigation, still uses the term labial glands, though he, too, is of the opinion that any comparison between reptilian and mammalian lips must be "zwar rein förmlich . . . die innere Bedeutung dieser Gebilde doch tatsächlich eine ganz andere ist."⁵⁰

In the adult *Aristelliger* the line of orifices of these glands in the lower jaw is freely exposed, so shallow is the labial groove as it runs between the row of teeth internally and the hard inflexible margin of the mandible externally. In the upper jaw a corresponding groove is scarcely perceptible. Barge places the allied *Hemidactylus* with lizards having a vestibulum oris, and hence lips, on both jaws, though in *Hemidactylus* very poorly developed. In *Gecko* he finds none at all.

⁴⁹ Bolk, L. *Odontological essays*. III. On the tooth-glands in reptiles and their rudiments in mammals. *Journ. of Anat.*, 1921, vol. 55, pp. 219-234 (pp. 230-234 here cited).

⁵⁰ *Loc. cit.*, p. 719.

In addition to certain lizards with no lips, with lips on both jaws, and with lips on the lower jaw only, Barge describes several with pseudovestibula, which are clefts separating what appears to be gingival tissue from the teeth,—“eine Spalte zwischen der nackten Kieferoberfläche und der Schleimhaut.” Although true lips rest against the ‘naked teeth’ and by suppression of gingival tissue would rest against the ‘naked jaw,’ Barge considers that a fold of tissue so situated cannot be a true lip—it is a ‘labiale Schleimhautfalte’ of gingival nature. In *Calotes jubatus* a cleft posterior to the teeth produces in each jaw a similar ‘linguale Schleimhautfalte’ (apparently inadvertently labeled ‘labiale’ in his Abb. 13, 14, and 15). These folds in the figures of *Calotes*, *Draco*, and especially of *Hatteria* suggest the internal lips or breathing-valves of teleosts, which, as in the toadfish and cod, bear a nodulose border pressed against the teeth anteriorly.

Barge’s subdivision of the tissue in front of the teeth, in *Calotes* and *Draco*, into a *pars gingivalis* between the pseudovestibulum and true vestibulum, and a *pars labialis* anterior to the true vestibulum, seems to correspond with our *pars villosa* and *pars glabra* in the cod, the *gingivalis* or *villosa* being molded against the teeth in both cases, and his terms may prove the better ones. In *Hatteria* he shows both parts as having indented crests; and toward the corners of the mouth, where the vestibulum is deepest, “es macht den Eindruck, als wäre die Lippe mit Papillen besetzt.” Altogether there is a most striking analogy between the conditions in *Hatteria* and the cod, as may be seen by comparing Barge’s figure 1 with our figure 2.

Ophidia

In *Typhlops*, a Cuban ‘blind snake,’ there is no depression comparable to a labial groove and consequently no lip-like fold on either jaw (fig. 40).⁵¹ Instead, a cornified epithelium

⁵¹ The adult specimens examined were recently collected in Cuba by Professor Bremer, who obtained also the *Aristelliger* embryos in Jamaica. I am indebted to him for the opportunity to report upon them.

extends well into the mouth, forming a sort of beak. This epithelium consists of two very distinct strata—an inner layer, with distinct cell walls, and an outer layer, less than half as thick, composed of homogeneous keratinized substance. The horny stratum, in sections, is often stripped away from the productive stratum beneath. Aborally it is continuous with the similar layer belonging to the cycloid scales which cover the body uniformly. Within the mouth, before reaching the transverse line of teeth in the upper jaw, it is interrupted by large glands, as shown in the figure; there are no teeth in the lower jaw.

Bothrops, as described by Barge, has a lower, but no upper lip; *Tropidonotus* and *Python* have both. In *Tropidonotus* he finds that the outer surface of the lip is scaly; the passage from skin to mucous membrane occurs along a sharp border; between the gingiva and this border there is a rather wide depression “which is to be considered a vestibulum oris”; in the floor of the vestibulum gland-outlets are present in a regular succession. In *Python*, lips are present anteriorly in both jaws,—“nach vorn . . . eine gut ausgebildete Lippe.” The vestibulum, which is Barge’s criterion of a lip, does not extend far posteriorly; and in the median line of the lower jaw it is interrupted by what might be called a frenulum.

Chelonia

Turtles, though Barge dismisses them with the comment, “Für eine Studie der Lippenbildung können diese Reptilien nicht in Betracht kommen,” present in the embryo a labial groove (Lippenfurche) identified and figured by Röse.⁵² His description is very brief, and his interpretation of the groove is justified only because it resembles closely the labial groove in birds. A single inverted drawing of the upper jaw in an advanced embryo of the green turtle (*Chelonia mydas*) accompanies Röse’s description.

⁵² Röse, C. Über die Zahnleiste und die Eischwiele der Sauropsiden. *Anat. Anz.*, 1892, Bd. 7, S. 754.

In older embryos of *Chrysemys marginata* (as in the 27-mm. specimen, fig. 35) this groove, running along the side of the upper jaw, may readily be identified. There is no corresponding groove on the lower jaw. It is placed somewhat above the epithelial thickening which is to form the horny beak, and is not merely a boundary of the cornified region. The beak crowns the maxillary bone, and medially ends at an epithelial thickening (fig. 35, *x*, corresponding with *y* on the lower jaw) which is apparently Röse's dental lamina (Zahnleiste). Fortunately, its interpretation is not within the field of the present inquiry. The labial groove becomes shallow and disappears posteriorly, and also anteriorly, for it does not cross the median line. It seems unquestionably comparable with the labial groove in birds, to be described in the following section.

The best evidence that the labial groove in turtles has been correctly interpreted is afforded by the 'Lippenschildkröten'—the group for which Geoffroy St.-Hilaire, in 1809, proposed the generic name *Trionyx*, remarking, "As to the true lips found in these turtles, it is a feature all the more surprising. . . ." A section of a newly hatched *Trionyx ferox* (fig. 36) shows these lips (*l.s.* and *l.i.*) to be extensive everted folds of vascular connective tissue, free from muscle. Their histological structure has been described in some detail by Hoffmann,⁵³ who could find no epithelial sense organs. Considering the relation of the upper lip to the tooth-like epithelial cornification (fig. 36, *a-b*), it seems clear that the lip arises above the labial sulcus, which is represented either by the 'pseudovestibulum' (*b*) or by the shallower, more lateral groove on the under side of the lip. We have not the intermediate stages needed to decide between these alternatives. On either side of the hard dental formations the soft tissue rises in a fold, as is so generally the case in the lower vertebrates.

Toward the corners of the mouth, in the adult, the everted condition of the upper lip comes to an end. The lip is then a

⁵³ In Bronn's *Thierreich*, Bd. 6, Abth. 3, Erste Hälfte, S. 233.

thin fold which descends below the lower lip and conceals its posterolateral portion, much as in *Necturus*. It does not, however, turn upward into the infralabial sulcus of the lower jaw. Both the upper and lower lips end abruptly before reaching the median line; and this, for the lower lip, is the condition in *Necturus*. But whereas the amphibian has a blunt snout, that of *Trionyx* is much prolonged, and the lips do not extend toward its tip.

BIRDS

"In birds the purposes of nutrition and defence are fulfilled by a bone-like beak, which forms a compound substitute for teeth and lips." Such was Aristotle's opinion.⁵⁴ In chicks about to hatch, Aldrovandi could add that on the tip of the upper bill, there is "something whitish, cartilaginous, and rather hard, round and like a millet-seed." He thought it guarded the tip like the button on a fencing foil, lest the beak puncture some veins, membranes, or other delicate structure, "and women say that chicks cannot pick up food until it has been removed."⁵⁵ But his artist overlooked this rostral callus when he drew the chick 'in utero,' as did Harvey also. In arguing against Fabricius, who thought that the hen broke open the egg on hearing the chick peep within it (—move within it, according to the Hippocratic writings),⁵⁶ Harvey

⁵⁴ *De partibus animalium*. Oxford ed., transl. by W. Ogle, 659^b 20. This reference and the discussion of the literature which follows have been borrowed from notes made by Dr. F. T. Lewis.

⁵⁵ *Ornithologiae tomus alter*, 1600, p. 218. Compare Bloch, *Abd. d. k. Leop.-Carol. Akad.*, 1904, Bd. 82, S. 307.

⁵⁶ An opinion which will not die. Réaumur (*Art de faire éclore*, etc., Paris, 1749, T. 1, pp. 311-342) accounts for it in three ways: the hen may have been seen removing fragments of shell from the nest; the eggshell may at first be broken as if from the outside, since the shell membrane can remain intact; the hen in turning the eggs may break them, a catastrophe. Sacc ('47) finds that the mother greatly aids the chick in getting out, by breaking carefully (*cassant avec précaution*) the shell around the aperture first made by the chick. Voeltzkow reported to the Berlin Academy (*Sitz.-ber.*, 1891, *Erster Halbband*, S. 115-120) that Madagascar crocodiles in the egg, buried 2 feet in the sand, make known by a sound audible 'the length of a room' their wish to be freed; whereupon the mother animal, which sleeps upon the nest, scrapes away the sand so that

was content to comment, "neither is the bill of the chick so soft, nor yet so far from the shell, that it can not pierce through the prison walls."⁵⁷ The first and, until Gardiner's, the best figures of this structure are those which John Hunter was arranging on the evening before his sudden death (in 1793).⁵⁸ His notes refer to the "little horny knob at the end of the beak with which the gosling breaks the shell" as if it were already known; but it was new to Yarrell in 1826, when he wrote *On the small horny appendage to the upper mandible*, describing it in pigeons, chicks, ducks, and geese. "Having performed the important office of dividing the shell, it is easily separated by the edge of the thumb nail of the attendant . . . or by the chicken itself in its early attempts to pick up food."⁵⁹ In Hunter's figures, and especially in those of Gardiner,⁶⁰ this horny appendage is remarkably mammiform, and is surmounted, in loco papillae, by a hard spine which feels like a pin-point.⁶¹ The callus rostralis, or from its function ruptor putaminis,⁶² culminates in a spina rostralis (seu spina ruptoris). It is shown in longitudinal and transverse section in figures 38 and 39, from chick embryos of 43 and 31 mm., respectively.

In 1841, Mayer reported that turtles and crocodiles have a callosity like that in birds.⁶³ It appears in the 26-mm.

the young can escape. This does not involve any breaking of the shell on the part of the mother, yet to Sluiter it seems 'almost fabulous.' Reese notes that 'like the alligator, the young crocodile makes a squeaking noise shortly before hatching and the mother doubtless opens the nest' (Alligator and its allies, 1915, p. 42).

⁵⁷ Works of Wm. Harvey. Transl. by Willis. Syd. Soc., p. 267.

⁵⁸ John Hunter's observations on animal development. Ed. by R. Owen, 1841, pl. 76, figs. 17, 18, et al., of the goose; also p. 31.

⁵⁹ Zool. Journ., 1826, vol. 2, p. 433-437.

⁶⁰ Gardiner, E. G. Beiträge zur Kenntniss des Epitrichiums und der Bildung des Vogelschnabels. Inaug. Diss., Leipzig, 1884, figs. 22 and 23.

⁶¹ "Welches sich wie eine Stecknadelspitze anfühlt"—Rosenstadt's apt description. Duval (Atlas, 1889, p. 47) notes that it is called 'le diamant,' referring presumably to the glass-cutter's implement.

⁶² Literally 'shell-breaker,' which in English is Heilmann's term for it. He found it in the heron, eider duck, avocet, coot (*Fulica*), and snowy owl (The origin of birds, London, 1926, pp. 94-95). It is also called the 'egg-pip.' Apparently there is no species of bird in which its absence has been established.

⁶³ Froriep's Notizen, Oct., 1841, Bd. 20, S. 70.

Chrysemys (fig. 37). Unfortunately, Mayer referred to this rostral callus as the 'egg-tooth' (Eizahn) in which he has been followed by uncritical writers, Röse and Sluiter protesting vigorously. Mayer asserted that the spina raptoris of the chick is sometimes double, and described it (when magnified four times) as consisting clearly of two pointed, conical, bright yellow crystals or teeth, placed quite near each other in pockets of the skin of the beak, from which they project obliquely outward toward either side. "They are not always equally developed, and sometimes only one is present." He does not describe this spina duplex as calcareous, but refers to dust from the shell found around it when an egg is opened after the chick has peeped; and he mentions that it 'rubs through' the shell.

Sacc, in 1847, was sure that "it is not through wearing away the shell, by rubbing it with its beak, that the chick breaks it open, but by striking it violently." He associated this act with respiration and raising the head. "The beak of chicks," he continues, "at the time of hatching is so weak, that it would be absolutely impossible to break the shell, if nature had not placed there this little calcareous tubercle, which becomes detached soon after birth: all the chicks which lack this outgrowth perish in the egg."⁶⁴ Similarly, Dr. Horner reported to the British Association in 1853,⁶⁵ "that the opinion that the shell is broken by a cutting or scraping motion of the bill, through the agency of the pointed horny scale at its end, is fallacious, as the membrane which lines the shell is at first left entire, while the shell itself outside has been chipped or broken off." He observed that the shell is broken generally "by a single smart blow only, though in some instances the blow is immediately repeated, or doubled; and each strike is made with considerable power, as is not only seen, but felt and heard." Keibel finds that the hypertrophied musculus complexus is adapted to thrust the rostral spine powerfully against the shell and to crack it, and is

⁶⁴ Sacc. Ann. des sci. nat., 3^e Sér., Zool., T. 8, p. 168. Paris. 1847.

⁶⁵ Brit. Ass. Adv. Sci., 1853, pp. 68-69.

thus the chief active agent in the process.⁶⁶ Other muscles may play a part.⁶⁷ The spine is serviceable in breaking the shell if it merely serves to concentrate the eruptive force at a point. As Professor P. W. Bridgman expresses it, "If the total amount of force which can be exerted upon the shell is fixed, the intensity of the counterforce in the shell must be greater if the action of the breaking force is confined to a small area."

Turtles use the rostral spine in making "a small rip at one end of the egg, which they enlarge by moving the head from side to side, sometimes aided by the claws of their forefeet."⁶⁸ They do not, like the chick, turn around within the egg, detaching a cap of shell, but come out through a terminal cleft. The difference which Weinland alleged, that in turtles the spine 'is gradually worn off,' whereas in birds it drops off, does not exist. Dr. H. L. Babcock informs us that in spotted turtles the spine may be spontaneously shed on the tenth day, at which time they have all become readily detachable. In crocodiles, according to Voeltzkow, the spine comes in contact with one end of the egg and by its mechanical operation, which 'genau wie ein Bohrer wirkt,' it pierces the shell.⁶⁹

Mayer's report that there are two diverging rostral spines in the chick is explicitly rejected by Gardiner and by Heilmann, though the latter found in the avocet a V-shaped base leading to the single apex. In three chick embryos of about fourteen days in the Harvard Collection, the structure is precisely and symmetrically median, with no suggestion of a paired origin, and the same appears to be true of turtles. But in the crocodiles it is double, as Mayer stated. Voeltzkow and Sluiter agree that in them it develops from two separate epithelial mounds. According to Voeltzkow, a little papilla

⁶⁶ Keibel, F. Wie zerbricht der ausschlüpfende Vogel die Eischale? *Anat. Anz.*, 1912, Bd. 41, S. 381-382.

⁶⁷ Pohlman. *Anat. Rec.*, 1919, vol. 17, pp. 89-104.

⁶⁸ Cochran, D. M. The box turtle. *Nature Magazine*, Sept., 1927, p. 152.

⁶⁹ Voeltzkow, A. *Sitz-ber. Akad. Wiss., Berlin*, 1891, Erster Halbband, S. 115-120; *Ann. Nat. Hist.*, 1892, vol. 9, pp. 66-72.

then arises between them, forming a bridge from one to the other, the end result being a symmetrically bifid median structure.⁷⁰ In the two specimens drawn in figure 5*B* and *C*, the entire spine is toward the left, and in one instance its right and left portions are quite unequally developed. The pit shown in figure 5*B* may indicate that the spine on the right has been suppressed, in which case the bifid structure would be wholly a left spine, but this single specimen by no means justifies so strange a conclusion.

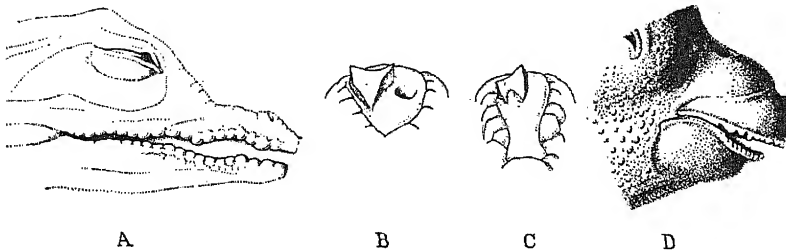


Fig. 5 *A*, head of an unhatched *Crocodilus americanus* (total length 133 mm.). $\times 2$. *B*, dorsal aspect of the raptor of the same embryo. $\times 12$. *C*, raptor of a 137-mm. *C. johnstoni*. $\times 12$. *D*, beak of a parrakeet embryo (*Melopsittacus undulatus*), after Braun, Arb. zool. Inst., Würzburg, 1882, Bd. 5, Taf. 8, Fig. 15. $\times 4$.

In 1857, Weinland reported that he had caught a young sandpiper (*Tringa pusilla*, Wils.) which "still wore the hard horny tubercle on the upper bill" and to his surprise he found "a similar armature on the lower bill, though less prominent." "I suppose," he continues, "that the horn on the lower bill serves only as a support for the upper bill while knocking."⁷¹ Unfortunately, Weinland did not fix the location of the structure on the lower bill. In a heron (*Ardea nycticorax*) about to hatch, which by chance was brought to the laboratory, the mandible is tipped with a well-defined pointed projection which lies within the trough of the upper bill when the beak is closed. It does not correspond in position with the raptor of the upper bill, which accordingly has no counterpart below; a slight diffuse swelling involving the symphysis seems irrelevant. But Rosenstadt, with no reference to Weinland, has recorded in cytological

⁷⁰ Voeltzkow, A. Abh. d. Senckenberg. Naturf. Ges., 1899, Bd. 26, S. 74-77.

⁷¹ Proc. Essex Inst., vol. 2, 1856-1860, pp. 115-116.

detail the production of a rudimentary rostral callus (Eizahnanlage) in chick embryos of fourteen to fifteen days, situated "in der mittleren Partie des Unterschnabels" and extending "in einen dünnen Streife auf beide Seiten."⁷² Its presence there confirms his opinion that even on the upper bill it is of no use in breaking the shell, being a formation without the slightest physiological significance. We have seen no indication of a rostral callus on the lower bill of chicks, which moreover lacks the pointed tip observed in the heron.

Functionally similar to the rostral callus of crocodiles, turtles, and birds, but structurally totally different, is the well-known egg tooth of snakes and lizards. In 1839, Johannes Müller reported to the Berlin Academy his discovery of a peculiar, flat or upturned, intermaxillary tooth, found at the time of hatching in snakes and lizards generally. He described it as growing forward beyond the line of the upper jaw, and apparently serving as a chisel to cut through the egg shell. But he found it equally developed in those species which bring forth their young alive.⁷³

In 1853, Dr. Weinland, of Cambridge, Massachusetts, a student of Müller's, observed the snake's egg tooth in action. It is a true incisor putaminis, or, from its rostral position, a dens rostralis, and produces "a long sharp slit through the thick leathery shell . . . cut as if by a sharp knife." Weinland made a microscopical preparation, which showed that this tooth consists of dentine with characteristic canaliculi, bounding a pulp cavity, and capped by a transparent, structureless layer which has since been described as enamel, but in which Weinland failed to find enamel prisms. It is a true tooth developed solely "to cut open the eggshell, which it may perform in the time of a second, and soon after it drops." Weinland discourses on Nature's elaborate preparations for so brief but important an event.⁷⁴ It remained for Sluiter to show that in its embryological development the shell incisor of lizards is a perfectly characteristic tooth. In Gecko, he found a pair of such teeth, close together, and so beveled that they combine to make a V-shaped apex.

⁷² Rosenstadt, B. Arch. f. mikr. Anat., 1912, Bd. 79, S. 632.

⁷³ Arch. f. Anat. Physiol. u. wiss. Med., Jahrg. 1841, S. 329-331, with a plate of excellent figures.

⁷⁴ Weinland. Proc. Essex Inst., Salem, vol. 2, pp. 28-32. But after 'cracking the shell' it takes the ringneck snake, *Diadophis*, nearly a day to emerge. It may withdraw its head and later project it through the same or a new opening, these openings being somewhat on the side of the egg and not quite apical. "Cracking the shell seems to be produced by the thrusting movements of the head or the turnings of the body of the snake. . . . A small eggtooth may be partly responsible for the first break in the shell." (Blanchard, F. N., Michigan Acad. of Sci., 1927, vol. 7, pp. 284-285.)

In *Mabuja* and *Lacerta*, he saw that the left member of the pair is small and unerupted, when the right is functional and so displaced as to appear median; and in *Calotes* and several snakes the median shell incisor is presumably a right one, with the left entirely suppressed.⁷⁵

Mayer correlated the spine of birds, turtles, and crocodiles with the calcareous egg shells in those groups, and the tooth of snakes and lizards with leathery or membranous shells. This opinion R  se adopted, but Sluiter ruled out, noting that many turtles produce eggs with leathery envelopes, and the eggs of some lizards have very hard, limy shells. It is important that no species has been found having both spine and tooth.

Recent writers agree that the rostral callus is non-calcified. It is a horny emergence of the subepitrichial epithelium, with no connective-tissue core. Figures and detailed histological descriptions have been supplied by Gardiner, and especially by Rosenstadt. Since it occurs within the area of greatly thickened epithelium bordering the oral aperture, where proliferative activity takes many forms, it might possibly have some relation to labial villi. It seems, however, to represent what in mammals would be the tip of the nose. That would mark the external boundary of the labial territory in birds. The internal boundary depends upon the position of the missing teeth.

In locating the dental area of birds, parrots have been particularly serviceable, "un heureux hasard ayant mis    la disposition de M. Geoffroy un f  tus de perroquet pr  s d'  clore." Geoffroy reported to the Acad  mie, in 1821, that he saw, along its beak, a row of tubercles "pr  sentant toutes les apparences ext  rieures des dents" (compare fig. 5*D*). Although these tubercles were not implanted in the bone of the jaw, there were canals in the bone, corresponding in number with the tubercles, which transmitted vessels and nerves to their gelatinous central pulps. In lieu of enamel, the crenulate margin of the beak was covered with a tenacious white transparent layer, passing continuously from one elevation to another, and suggesting to Geoffroy the compound

⁷⁵ Sluiter.   ber den Eizahn und die Eischwiele einiger Reptilien. *Morph. Jahrb.*, 1893, Bd. 20, S. 75-89.

tooth of the elephant! Soon after hatching, progressive cornification obliterated the tubercles, but the permanent serrations in the bills of certain ducks were considered comparable.⁷⁶

Since that time, parrot embryos have been studied frequently. E. Blanchard, in cockatoos, thought he saw dentine and bony sockets for the tubercles;⁷⁷ Fraisse, in parrakeets, found no trace of dentine, and no alveoli. The papillae are very vascular, and may become elongated, so that after removal of the horny layer they are soft and float about in the fluid in which they are examined⁷⁸—an observation suggesting what happens with human labial villi. Fraisse reasserts that the horny teeth of the mergansers are comparable formations. Braun's studies provided the excellent figure here copied in part as figure 5D.⁷⁹ Gardiner thought it 'possible but far from probable' that the elevations were due mechanically to the beak becoming hooked; but Ihde shows that this is a futile suggestion.⁸⁰ Gardiner more reasonably stated (l.c., p. 44) that the papillae in the parrot may functionally be correlated with the production of the horny beak, since they resemble the papillae which he found in hoofs. In the beaks of embryo chicks, ducks, pigeons, and hawks he found papillae similar to those of the parrakeet, except that they failed to produce nodular elevations. In the duck he recorded only a single row in the upper jaw, but three or four rows in the lower. In the parrot additional small nodules occur on the roof of the mouth. Fürbinger, who reviewed the literature and sought for tubercles in the Laridae and Limicolae, concludes that in recent birds "elevations like dental papillae arise, largest apparently in the parrots," but they

⁷⁶ Geoffroy St.-Hilaire. *Mém. et Hist. de l'Acad. des Sci.*, Année 1821, Paris, 1826. *Analyse des travaux* (par Cuvier), pp. 189-191.

⁷⁷ Blanchard, Émile. *Comptes rendus de l'Acad. des Sci.*, Paris, 1860, T. 50, pp. 540-542.

⁷⁸ Fraisse, P. *Verh. d. phys.-med. Ges.*, Würzburg, 1881, Bd. 15 (Sitzber. für 1879-80, S. iii-vi).

⁷⁹ Braun, M. *Die Entwicklung des Wellenpapagei's* (*Melopsittacus undulatus*). *Arb., zool.-zoot. Inst.*, Würzburg, 1882, Bd. 5, S. 178-179, Taf. 8, Fig. 15.

⁸⁰ Ihde. *Arch. f. mikr. Anat.*, 1912, Bd. 79, S. 255.

are destitute both of enamel epithelium and of dentinal cells.⁸¹

As seen by comparing figure 5A with 5D, Geoffroy's dental tubercles of the parrot closely resemble the alveolar mounds along the jaws of the crocodile, through each of which a tooth is soon to emerge.⁸² It seems probable, therefore, that the tubercles in the parrot indicate the position of the missing teeth. The dental lamina of Röse is, however, immediately internal to their bases, and therefore quite separate from them (as may be inferred from fig. 39). Ihde has marshaled the evidence of those who question or deny the dental nature of this epithelial proliferation or 'lamina.'

Above the row of rostral tubercles shown in figure 5D, there is a groove, which Gardiner, of Boston, in his Leipzig dissertation, interpreted as a labial groove (*Lippenfurche*). Röse comments: "Nach meinen Untersuchungen ist es zweifellos, dass Gardiner sehr im Rechte ist, wenn er in dieser Furchen ein Analagon der Lippenfurchen vermutet."⁸³ Ihde, as critic, finds that 'Homologon'—not 'Analagon'—should have been Röse's term, and continues:

Ob die Furchen tatsächlich eine rudimentäre Lippenfurchen ist, weiss ich nicht. Sie liegt im Obersehnabel beim Hühnchen und Papagei auswärts vom Schnabelrande und ist noch in vorgerückten Alter des Tieres nachweisbar. Die Deutung als Lippenfurchen ist eine rein willkürliche und solange haltbar, als keine bessere da ist, wobei ich allerdings gestehen muss, dass mir eine bessere und wahrscheinlichere nicht bekannt ist.⁸⁴

Gardiner's labial groove and Röse's dental lamina (or sulcus) are readily found in sections of the chick (fig. 39). Although the labial groove should be on the under surface of the upper jaw and directed upward (as in lizards and snakes), its lateral position in crocodiles and birds is not as fatal to

⁸¹ Fürbinger, M. *Unters. z. Morph. u. Syst. d. Vögel*, Amsterdam, 1888, Bd. 2, S. 1074-1075.

⁸² Röse has figured the toothed embryo of *Crocodylus biporcatus* in the *Anat. Anz.*, 1892, Bd. 7, S. 757.

⁸³ Röse, l.c., S. 753.

⁸⁴ Ihde, l.c., S. 270.

homology as might be supposed. For in the upper jaw of a chick of 4.5 mm. the labial groove is ventral in its hinder part, shifting to a lateral position anteriorly. It increases in depth toward the point where it crosses the median line. In *Tritonyx* the upper lip unquestionably is in relation with a corresponding groove. The relations in the lower jaw of the chick are similar to those in the upper, though the dental lamina is hardly appreciable. The lower labial groove also is deep anteriorly, and the epithelium between it and the oral cavity is invaded by a row of large papillae, quite round in transverse section, which clearly represent the tubercles of the parakeet. As seen without magnification on the beaks of chicks toward the time of hatching, it certainly marks off a lip-like area of the horny beak.

Accepting the labial groove as defining the inner margin of the lip, the labial territory of birds becomes the exterior of the cornified beak. Histologically, it suggests the mammalian lip only in the remarkable thickness of its epithelium.

MAMMALS

Monotremes

In the absence of suitable specimens of either duck-bill or echidna, which lack lips according to Wiedersheim, Kopsch, and Schumacher (as noted in our 'Introduction'), the following citations may be of interest. Burrell finds that the oral integument of the duck-bill becomes horny and like a bird's beak only in dried specimens. He describes it in life as soft and moist, and supplied with innumerable tactile corpuscles—"the most sensitive portion is undoubtedly the anterior border of the upper lip." He defines the lips of the duck-bill as that "pliable tissue extending beyond the jaw bones," and states that the lips may be puckered to the extent of forming a small central suction tube.⁸⁵

In *Echidna*, Ruge, who makes no mention of lips, describes the anterior fibers of the buccinator muscle as assuming the

⁸⁵ Burrell, H. *The Platypus*, Sydney, 1927, pp. 9-10 and 68.

character of a sphincter oris, though divided very definitely into lateral halves.⁸⁶ But Owen saw "lips transitorily manifested at the suckling period"; in the adult they become reduced to the scarcely movable margin of the small terminal oral orifice.⁸⁷

On the tip of the snout of *Echidna*, Semon found "an epidermal thickening—a small projecting mound—which certainly, as in *Sauropsida*, plays a rôle in breaking the eggshell." "After hatching it gradually vanishes."⁸⁸ But here it is in nasal, and not in labial, territory. In the duck-bill Owen had described, "on the middle line of the upper mandible and a little anterior to the nostrils, a minute fleshy eminence . . . of which the adult presents no trace . . . obviously analogous to the horny knob of birds."⁸⁹ Burrell thinks that a ruptor is not needed by the restless muscular embryo within so thin a shell, and finding that the caruncle is at first soft, becoming hard and sharp a couple of weeks after hatching, he conjectures that it may serve as a 'milk-spur' to incite lactation (i.e., p. 185).

Marsupials

Apparently all marsupials have lips, the muzzle generally being red from the vascularity of the integument (Owen). The lips of thirty-seven species have been studied macroscopically for elevations by F. E. Schulze,⁹⁰ whose propensity for Greek nomenclature is suggestive of Heusinger's. In the kangaroos the vestibulum ('chilocoele') extends back laterally to a transverse fold—the 'crista transversa superior' in the upper jaw and 'crista transversa inferior' in the lower. These folds cross the vestibulum in the interval between the cutting teeth and the molars, and tend to divide the mouth into anterior and posterior chambers. The posterior portion of the vestibulum, passing into the cavity of the cheeks, is the

⁸⁶ Ruge, G. Die Hautmuskulatur der Monotremen. *Jenaische Denkschriften*, V (Semon, *Zool. Forschungsreisen*, ii), 1895, S. 135.

⁸⁷ *Comp. Anat.*, vol. 3, p. 383.

⁸⁸ Semon, R. *Jenaische Denkschr.*, V, 1894, S. 73.

⁸⁹ *Trans. Zool. Soc., London*, 1835, vol. 1, p. 224.

⁹⁰ Schulze, F. E. Die Erhebungen auf der Lippen- und Wangenschleimhaut der Säugetiere. II. Die Beuteltiergattung *Macropus*. III. *Marsupialia*. *Sitz.-ber. d. Akad. d. Wiss., Berlin*, 1913, S. 384–395; 1916, S. 43–65.

'pariocoel.' The lips are hairy externally, with the upper divided into right and left halves by an interval of bare skin.⁹¹ Each lip may have an "evenly rounded, smooth outer margin" (with shallow indentations laterally, or sometimes conical or hooked papillae) which is perhaps a pars glabra—the 'ektochil' of Schulze. Internally, in place of a villosa, Schulze finds two partly overlapping ridges—'entochil' and 'parachil,' respectively—the former nodular, and the latter comb-like with a row of pointed horny spines. At the angle of the mouth the nodular ridges of upper and lower lip meet, and are continued along the inside of the cheek as a single row of nodules for a short distance, when the row divides into an upper and a lower branch. Between them there is sometimes a velvety area of small papillae. This is the general plan in marsupials, the many deviations from which, in various genera, are clearly shown in Schulze's drawings and photographs.

The development of the marsupial lip, which Schulze did not consider, is shown in figure 55—a sagittal section of a 23-mm. opossum from the pouch, including the maternal nipple in its mouth. The dental lamina grows down behind the bone of the lower jaw, and the labial lamina in front of it, so like the condition in lizards (fig. 34), which in turn is like that of sharks, that we conclude that all of these animals have at least the beginning of true lips. In the upper jaw of the small opossum there is a marked epithelial thickening, which extends beyond the limits of the ill-defined labial lamina, and forms a projecting shelf of cornified cells pressed against the nipple. With this apparatus, at a stage before characteristic mammalian lips have developed, nursing is very successfully accomplished.

At a length of 170 mm., when the opossum is about ready to shift for itself, there is still no upper lip adjacent to the

⁹¹ Schulze states that this bare median groove, present and reaching the nares in some, but by no means in all, the herbivorous marsupials, can have no other significance than to convey the nasal secretion to the mouth to mix with the saliva (l.c., 1916, p. 65). This is precisely the unlovely method of the camel to conserve moisture, as described by Owen (Comp. Anat., vol. 3, p. 393).

median line. There the thickened epithelium extends continuously from the anterior portion of the palate to the exposed part of the snout. But laterally there is a deep labial groove or vestibulum, and toward the corners of the mouth all the features of typical mammalian lips are present (fig. 56). The hairy skin (pars cutanea) becomes a pars glabra at the outer edge of the lips, and this forms a very thick pars villosa, invaded by slender connective-tissue papillae on approaching the vestibule. The free surface of the villosa of the upper lip is merely corrugated, but on either half of the lower lip, it bears a row of some forty conical projections, about 0.25 mm. tall. There are a few low nodules within the cheeks. Each lip is plentifully supplied with striated muscle.

Edentates

The anteaters have small mouths in which "introduced termites may be crushed by the action of the tongue against two callous ridges, which seem to occupy the place of teeth." The tongue is their prehensile organ, but their lips are muscular. In *Myrmecophaga jubata*, Owen has dissected the orbicularis oris and the retractors of both lips.⁹² In *Manis pentadactyla*, Carus and Otto have figured the upper lip as extending well below the lower lip at the corners of the mouth⁹³—a feature of the lips in *Trionyx* and *Necturus*. Many hairs directed backward are said to occur within the cheeks.

Ungulates

The lips of the pig have no villous or papillary outgrowths. The pars villosa is a band of thickened epithelium invaded by tall vascular dermal papillae (fig. 48, from a 182-mm. embryo).

In ruminants, free villi and perhaps the entire lips attain their maximum development. The moose, writes Gilbert White, of Selborne, has "such a redundancy of upper lip as

⁹² Trans. Zool. Soc., London, 1862, vol. 4, p. 133.

⁹³ Carus, C. G., and Otto, A. W. *Tabulae anatomiam comparativam illustrantes*. Pars IV. Lipsiae, 1835, S. 14 and Taf. 7, Fig. 6.

I never saw before.”⁹⁴ The macroscopic and well-known villi extend in a broad band from the angles of the mouth along the inside of the cheek, and serve ‘as mechanical obstacles’ to the escape of regurgitated food, confining the soft slimy mass to the molar region during the second mastication. “Neither the hog nor the horse have such buccal papillae” (Owen). Schulze has studied their function more recently.⁹⁵ In the adult sheep they are shown in figure 68, and a section of those in the calf in figure 69. If the villi are followed from the cheeks to the lips in either animal, and the attempt made to identify a pars villosa and pars glabra, the following complication will be observed. The villi, replaced by low nodules, extend to the hairy skin and eliminate any pars glabra (compare fig. 49). The area thus occupied is quite extensive, since it spreads over the labial margin into what, in other animals, is a pars cutanea. In the Bovidae and Cervidae the labial texture extends broadly to the nose, forming the characteristic muzzle or ‘Flotzmaul,’ for which there appears to be no English name.

Sirenians

The lips of the manatee have been described and photographed by Murie,⁹⁶ and their action in a living specimen has been recorded by Garrod,⁹⁷ but without making clear the limits of villosa and glabra, if such subdivisions exist. The ‘comparatively insignificant lower lip’ has a relatively smooth outer portion “with its sinuous, bristle-clad, thick epidermis,” succeeded within the mouth by a papillose area which some have termed an ‘inner lip.’ “In front, the inner

⁹⁴ The elephant may here be a competitor, since the upper lip, blended with the nose, forms the trunk. Owen found that the muscles of the trunk are a development of the orbicularis oris. “The under lip of the elephant alone is free and is produced into a pointed form.”

⁹⁵ Schulze, F. E. Die Erhebungen u. s. w. I. Ruminantia. Sitz.-ber. d. Akad. d. Wiss., Berlin, 1912, S. 510-521.

⁹⁶ Murie, J. On the form and structure of the manatee (*Manatus americanus*). Trans. Zool. Soc., London, 1874, vol. 8, pp. 133-134 and 164-166.

⁹⁷ Garrod, A. H. Notes on the manatee. Trans. Zool. Soc., London, 1879, vol. 10, pp. 138-139.

lip is separated from the outer by a deepish furrow, and behind it stops short at the tip of the tongue, though it is continuous with the gums" (Murie). Since the incisors never erupt in the manatee, and since the epithelium of the inner lip is thinner than that of the outer (Murie), the papillose mandibular pad or 'inner lip' is presumably gingival tissue.

The great upper lip is bilobed, with a median notch crossed by 'innumerable transverse muscle fibres' (Garrod). Through their agency, the contiguous bristly surfaces of the pendent lateral lobes may be brought together, seizing between them some portion of vegetable food, which is then drawn upward by a backward movement of the lower margin of the lip as a whole. The lobes are so vascular as to suggest erectile tissue. A somewhat horseshoe-shaped elastic pad is found on the roof of the mouth internal to the labial notch, which is studded with short erect papillae of two sorts—larger conical ones about half an inch high, with smaller setose forms in the interstices (Murie). This 'inner lip' is presumably gingival, and the 'deep furrow' between it and the 'outer lip' is then a vestibulum oris.

Not only are the lips bristly, but hairs are found within the mouth, where, as Murie suggests, they may be the homologues of the whalebone of some Cetacea.

Cetaceans

According to Kükenthal,⁹⁸ the form of cetacean lips is wholly dependent upon their physiological function. "In the first place they must effect a firm, tight closure of the mouth, preventing the entrance of water: and further, they must serve in obtaining food, in two ways, first,—in securing the mother's milk without admixture of water; and secondly, in the capture of edible organisms." He shows, in diagrams, how the concave border of the upper lip in the toothed whales is mortised, as it were, against the convex upper edge of the lower lip when the mouth is closed. In the whalebone whales

⁹⁸ Kükenthal, W. *Jenaische Denkschriften*, 1893, Bd. 3, S. 317-322.

this arrangement is reversed: the upper lip has a convexity which fits into a concavity of the lower lip. "It falls down like a thick curtain some feet in depth" (Owen). Kükenthal describes the labial sulcus as present in some species laterally, but not medially; and in the lower jaw of a *Balaenoptera musculus* he finds something suggestive of the 'false vestibule' of reptiles—"Eine eigentlich Lippenfurche, welche die Unterlippe von dem Kiefer trennt, fehlt, sie fällt zusammen mit der Kieferfurche, an deren Grunde die Zahnreihe liegt. . . . Im Oberkiefer ist eine Lippenfurche dagegen vorhanden" (i.e., p. 321). In *Hyperoodon rostratus*, which has horny formations in place of teeth, the gingival mounds meet tightly when the mouth is closed and the rudimentary lips cannot be brought together. Nothing is said of villi in any species.

Carnivores

The bilateral clusters of tooth-like villi within the upper lip of the cat have been the subject of a previous paper.⁹⁹ These villi are covered with thickened epithelium which extends laterally, but without villi, toward the corners of the mouth, assuming a position on the free margin of the lips. There, in advanced embryos (of 42.5 mm., fig. 46), it may form tag-like masses, sometimes pedunculated, which contained epithelial pearls. In the newborn (fig. 47) the thickened band is still present; and it may be recognized, though it is not well marked, in older cats. The *pars villosa* of the upper lip is thus represented laterally by a non-villous zone of greatly thickened epithelium. The lower lip has a similar zone which is altogether without villi. From this '*pars villosa*,' the *pars glabra* with thinner epithelium, is separated by a deep sulcus, characteristic of the carnivorous lower lip (fig. 44, *s.e.l.*). In figure 45, farther toward the corner of the mouth, there is an accessory sulcus, so that the entire section resembles Schulze's schematic figure of the lip in the kangaroo, with grooves separating *ektotchil*, *entotchil*, and *parachil* (Schulze, '13, p. 387).

⁹⁹ Anson, B. J. 'Denticles' . . . on the inner surface of the lip of the cat. *Anat. Rec.*, 1925, vol. 31, pp. 93-121.

In the newborn dog a band of thickened epithelium along the lower lip, in the same position as that in the cat, actually bears a row of villi, confirming its interpretation as a *pars villosa*. The villi, fifteen to twenty on each half of the lip, extend from the corner of the mouth halfway to the midline. Most of them are shaped like human incisors. Some broad ones near the medial end of the series are bifid; a few, laterally placed, are conical. Structurally, they resemble those of the upper lip of the cat. As seen from the outside, the entire villous crest, with its thick epithelium, is set off from the *pars glabra* below by a sulcus, as in the cat. The sporadic occurrence of nodular or low spinous excrescences over any part of the villous zone, as far as this sulcus, emphasizes the distinction between the two areas. In adult dogs the villi are smaller and apparently fewer than at birth. On the upper lip there are no villi at any stage, though in places the zone of thickened epithelium is crossed by furrows, producing a pebbled surface. In newborn dogs there are a few gingival villi, frequently constricted at the base, in relation with the cheek teeth.

Rodents

Flower and Lydekker¹⁰⁰ describe the mouth of rodents as "divided into two cavities communicating by a restricted orifice, an anterior one containing the large incisors, and a posterior one in which the molars are placed." Across the diastema between the incisors and molars the hairy integument of the face is continued into the mouth and meets the sides of the tongue. "This peculiar arrangement evidently prevents substances not intended for food from entering the molar chamber, as when the animal is engaged in gnawing through an obstacle." "In the hares and pacas the inside of the cheeks is hairy, and in the pouched rats and hamsters there are large internal cheek pouches lined with hairy integument." But Schulze, who names the hairy area *inflexum pellitum*, as if it were infolded skin of the cheek, suggests

¹⁰⁰ Introduction to the study of mammals. London, 1891, p. 446.

that the backwardly directed hairs may be useful, not in keeping things out of the molar chamber, but in guiding food into it.¹⁰¹ The lips are in relation with the anterior chamber. If the cheeks have developed by the fusion of the posterior parts of the lips, it may explain Schulze's observation that rodent lips lack the subdivision into anterior and posterior parts which he found in marsupials.

A vertical section through the lower lip of a newborn guinea-pig is shown in figure 51. The vestibule is deep, corresponding with the length of the incisors. The ill-defined pars glabra is hairy, as is the villosa. The epithelium becomes gradually thickened, and develops tall dermal papillae, but is without projecting villi. These features are more pronounced in a horizontal section through the cheek at the angle of the mouth (fig. 52). The transitional zone seems to have the character of hairy mucous membrane rather than of infolded skin, for its epithelium is three times thicker than the epidermis and becomes, at its posterior limit, twelve times as thick.

Embryologically, the lips of the guinea-pig develop from massive epithelial proliferations which bifurcate into dental and labial laminae (fig. 53, 18.6-mm. specimen). The direction of the lower labial lamina already indicates an incurved lower lip such as is found at birth (fig. 51). The lamina for the upper lip also splits very early, so that at birth both lips are well developed. In the rat and mouse, however, although the adult mouth resembles that of the guinea-pig, the labial laminae of the newborn are still unsplit. The young animals have no lips (fig. 54); thick epithelium fills what would otherwise be a labial sulcus and extends inward over the teeth, forming a considerable mound over the lower incisors.

The absence of lips in the newborn opossum and rat is doubtless correlated with the immature condition of their young at birth. It cannot depend on the length of the

¹⁰¹ Schulze, F. E. Die Erhebungen u. s. w. IV. Rodentia duplicidentata. Sitzber. d. Akad. d. Wiss., Berlin, 1916, S. 781; and V. Rodentia simplicidentata, *ibid.*, S. 1233.

maternal nipple—very long in the opossum and short in the rat—though Neustätter suggests that the special development of the human labial margin is because “die Warze beim Weib relativ viel kürzer ist als bei den Tieren, selbst den Affen.”¹⁰² The guinea-pig, with long nipples, has young with well-defined lips at birth, being in all respects far advanced at that stage. The rabbit is intermediate between guinea-pig and rat. Incomplete labial grooves are present. In the lower jaw, toward the corners of the mouth, there is no vestibulum oris and the lip is very ill-defined (fig. 50).

Bats

A mouth of unusual interest is that of the Mexican vampire—a *Phyllostoma*—figured by Carus and Otto.¹⁰³ The upper lip, along the external part of its margin, is beset with a great many soft, round, fleshy papillae, which become gradually larger toward the corners of the mouth. These are followed, along the inner margin on either side, by many long, soft spines or laminae, with apices directed toward the teeth; and in the region of the first molar there are several lesser fleshy papillae, likewise directed inward. The lower lip also has macroscopic soft round tubercles along its outer margin, and sharp, inwardly directed laminae internal to them; in the median line there is a larger tubercle, and below it a crescentic series of soft fleshy papillae. A large undulate fold of mucous membrane between the lip and mandible on either side completes the list of villous structures enumerated by the authors cited. Functionally, they state that these lips effect a firm and air-tight application of the mouth to the bitten skin of some animal, and serve as a cupping-glass in sucking blood.

¹⁰² Neustätter, O. Ueber den Lippensaum beim Menschen. *Jenaische Zeitschr.*, 1895, Bd. 29, S. 385.

¹⁰³ *Loc. cit.* (in footnote 93), Tab. VII, fig. 1, and S. 12.

Man

First we review the embryological features and then those of the adult. The mouth of the human embryo of 10.2 mm. in sagittal section (fig. 41) is readily comparable with that of the 22-mm. *Squalus* (fig. 11) or 12.8-mm. *Torpedo* (fig. 15). All are without lips and in all the mouth is ventrally placed—in man far from its ultimate position. This was well recognized by Balfour, who concluded that the vertebrate mouth was of suctorial derivation (Comp. Embr., vol. 2, pp. 263–264). We find this position correlated with functional palatine teeth and primary lips; and since such teeth do not develop in man, a forward migration of the mandible may be expected, causing the ectoderm to extend much farther along the roof of the oral cavity than along its floor. This migration is occurring in the 22-mm. embryo (fig. 42) in which the dental laminae are shown, and, in the lower jaw, the labial lamina. The approximation of labial and dental laminae at this stage in mammalian embryos produces what has been called the labio-dental plate. The union of the two is massive in the guinea-pig (fig. 53). In the lizard *Aristelliger* (fig. 34), the labial and dental laminae of the lower jaw are close together, but do not unite; in the elasmobranchs there is a considerable interval between them. In the human embryo of 70 mm. (fig. 43) the labial lamina grows down toward the front of Meckel's cartilage, and the dental lamina behind it, precisely as in the lizard, amphibians, and elasmobranchs; and on this simple but convincing evidence we base the conclusion that these lower vertebrates possess true lips. The varying relation of the lips to the median nasal and maxillary processes we regard as secondary differences due to variations in the location and development of the nasal pits. Consequently, it is not justifiable, from our point of view, to make the homologies of lips depend upon their relationship to those processes (His, Keibel, et al.).

The most recent description of human lips at birth is that of Schumacher,¹⁰⁴ in which we find confirmation of our

¹⁰⁴ Hdb. d. mikr. Anat. d. Mensch., herausgegeben von v. Möllendorff, Bd. 5, Teil 1, Berlin, 1927, S. 9.

observations that the lips of living infants lack villi. His guarded expression is as follows:

Jedenfalls geht aus den Beschreibungen und auch aus meinen eigenen Beobachtungen hervor, dass die Ausbildung der „Lippenzotten“ des Neugeborenen sehr grossen individuellen Schwankungen unterworfen ist. Mitunter ist von Vorragungen an der Oberfläche überhaupt nichts zu sehen. . . .

At the suggestion of Dr. Lewis, who made similar observations some years ago, I examined the lips of newborn and somewhat older infants in the Boston Lying-in Hospital, and have been unable to see the villi with the unaided eye. By careful examination with a hand lens (magnifying 8 diam.), when the lips were rolled outward, the mucous membrane was seen to be studded with minute red points of low conical shape. The red dots, or striae, doubtless represent the tips of capillary loops in the connective tissue of the slender dermal papillae. In infants of about one week the vascular dots seem to be replaced by minute whitish eminences, which result perhaps from a drying, and an increasing though slight opacity, of the overlying epithelium.¹⁰⁵

The epithelium over the labial papillae macerates readily in a peculiar way. In an infant at full term which died at birth, Dr. Lewis found the dark red inner part of the lip sharply marked off from the pars glabra, but no villi could be seen in the fresh preparation. There were low rounded mounds, or at best low conical elevations, in the pars villosa.

¹⁰⁵ These papillae are similar to those of the gums which were described by Henry Goadby in 1858, in a citation which Dr. Lewis has provided, from what he regards as the first original histology published in the United States ("A text-book of vegetable and animal physiology," New York, 1858). Goadby has a curious colored figure of the injected mucous membrane of the human gums. Its papillae in the adult, he says, "are somewhat long, and consist of a single looped capillary, which, through the tension of the injection, becomes more or less twisted. Their number is very considerable, presenting literally a forest of them. The papillae of the lips present a gorgeous sight, when well injected; the loops, as in the former case, twisting with the pressure of the injection." These loops, however, are not all as simple as Goadby showed them. They often include one or more anastomosing vessels, though the network is never as elaborate as in intestinal villi.

After hardening thirty-six hours in Zenker's fluid, sections of the left upper lip, near the corner of the mouth, showed distinct villi, as seen in the photograph, figure 67. Maceration of the lips from the opposite side of the mouth for twenty-four hours in Ringer's solution showed much taller and more slender villi, unlike anything seen in life, photographed in figure 66. After continuing the maceration for forty-eight hours, the epithelium came away from the villi in flakes, leaving the cores very prominent. These connective-tissue cores, even when slender, are quite resistant. Toward the pars glabra the epithelium was more tenacious, and over the glabra and skin it would not brush off, nor could it be detached with forceps as a layer in such a way as to display the papillae beneath.

Although the possibility of individual variation remains, so that some human lips may show distinct macroscopic villi in life, they have not yet been clearly recorded by any one, so far as we know. The figure of the human lips by Miss Ramm,¹⁰⁶ reproduced by Schumacher and in Keibel and Mall's *Embryology*, presumably represents a stage in the shrinkage or desquamation of the epithelium. It greatly resembles Ruysch's historic figure, the manner of the production of which he well understood. Consequently, it had been far better to name the zone in question the pars papillosa rather than the pars villosa, or from the proliferative abundance of its epithelium, the pars epitheliosa. However, the localized peaks of epithelial cells above the dermal papillae, such as are shown in sections of human lips, may readily pass into macroscopic elevations like those of many mammals. A *Semnopithecus entellus* at term has a very thick epitheliosa without villi over most of the lips, but there are a few free villi near the corner of the mouth. The lips of an adult *Macacus rhesus* have no free villi; there is a thick epitheliosa, as in the human adult.

¹⁰⁶ Ramm, M. Ueber die Zotten der Mundlippen beim Neugeborenen. *Anat. Hefte*, Abth. 1, 1905, Bd. 29, Fig. 2, S. 74.

CONCLUSIONS

1. We agree with Danforth¹⁰⁷ that "homology may be regarded as a purely relative matter. . . . It is in the causal factors and not in the structures themselves that the real basis of homology is to be sought. . . . Homology is consequently usually partial and not absolute." On such a basis we find homologous lips at certain stages of development in some representatives of all classes of vertebrates.

2. Kükenthal's statement concerning the lips of whales—*Die Form der Lippen ist vollkommen abhängig von ihrer physiologischen Function*—applies, with but slight restriction, to the lips of all vertebrates.

3. The lips of elasmobranchs are 'primary lips.' We qualify Allis's statement that "in the Amniota the functional lips are the secondary ones" by pointing out that the secondary lips in the upper and lower jaws are morphologically very different structures. In the upper jaw the secondary lip is a small subdivision of the primary lip. In the lower jaw the primary lip is suppressed altogether, or wholly merged in the secondary lip, correlated with the forward movement of the mandible and the persistence of the mandibular teeth. But we agree with Allis that "maxillary and premaxillary teeth or bones may be developed, as in the Teleostei, Holostei, and Crossopterygii, in relation to this secondary upper lip." "In most of the Sauropsida both of these latter bones are actually developed in relation to this lip, and there are, accordingly, in the upper jaw of these vertebrates, two arcades, with or without teeth, an inner and primary arcade formed by the bones developed in relation to the palatoquadrate and an outer and secondary arcade formed by the maxillary and premaxillary bones."

4. Since the upper teeth of the higher vertebrates are not represented in the elasmobranchs, but are a new formation within the territory of the primary lip, their derivation from placoid scales is secondary and remote. There is merely the

¹⁰⁷ Danforth, C. H. Hair in its relation to questions of homology and phylogeny. *Am. Jour. Anat.*, 1925, vol. 36, pp. 66-67.

slightest morphological resemblance between labial villi and denticles. Yet if the tooth-like labial villi of the cat, for example, should become teeth, and bone should develop in the lip in relation with them, the process of evolution of the secondary upper lip would only be carried a step further, leading to the production of a tertiary jaw and lip.

5. The functions of the vertebrate lip are three—sensory, prehensile, and adhesive—which are developed in varying degrees. Of the lips studied, those of the cod have the most conspicuous sense organs; those of the tadpole with their epithelial teeth, and of grazing animals through their intrinsic muscles, are the most prehensile; the lips of *petromyzon* and of the vampire, with abundant villi, are the most adhesive, and indicate that the smaller villi of the lips of suckling animals, together with their very vascular papillae, are for tight adhesion to the nipple. However, the fact that nursing may be accomplished without this apparatus and even before lips have developed, as in the opossum and rat, suggests an excess of ingenuity—an over refinement in nature—like that which produced the rostral callus, often considered unnecessary for breaking the eggshell.

6. Morphologically, the entrance to the mouth of vertebrates tends to be guarded by a pair of folds over which the skin makes a transition to the mucous membrane. This transition is effected typically through two zones, an outer *pars glabra* and an inner *pars villosa* (or *epitheliosa*). The latter is a region of very thick epithelium and of tall papillae of the corium, and is the seat of great proliferative activity, leading to the production of various papillae and villi, both in the lower vertebrates and in mammals.

PLATES

ABBREVIATIONS

<i>call.</i> , callus	<i>l.s.</i> , labium superius
<i>ch.</i> , chorda dorsalis	<i>mand.</i> , mandibula
<i>cir.</i> , cirrus	<i>m.g-h.</i> , musculus geniohyoideus
<i>c.M.</i> , cartilago Meekelii	<i>m.l.</i> , musculus labialis
<i>c.p.</i> , cartilago palatina	<i>m.ph.</i> , membrana pharyngea
<i>c.p-mn.</i> , cartilago premandibularis	<i>os.mand.</i> , os mandibulae
<i>c.p-qu.</i> , cartilago palatoquadrata	<i>p.cut.</i> , pars cutanea
<i>c.r.i.</i> , cartilago rostralis inferior	<i>p.glab.</i> , pars glabra
<i>c.r.s.</i> , cartilago rostralis superior	<i>p.hyp.</i> , pedunculus hypophyseos
<i>d.corn.</i> , dens corneus	<i>p.mam.</i> , papilla mammae
<i>d.lin.</i> , dens lingualis	<i>p.mx.</i> , premaxilla
<i>d.mn.</i> , dens mandibularis	<i>p.vil.</i> , pars villosa
<i>d.mx.</i> , dens maxillaris	<i>rost.</i> , rostrum
<i>d.pal.</i> , dens palatinus	<i>r.put.</i> , ruptor putaminis
<i>d.p-mx.</i> , dens premaxillaris	<i>s.dent.</i> , sulcus dentalis
<i>d.v-p.</i> , dens vomeropalatinus	<i>s.e.l.</i> , sulcus externus labii
<i>f.hyp.</i> , fovea hypophyseos	<i>s.i-l.</i> , sulcus infralabialis
<i>f.o.</i> , fissura oris	<i>s.i-m.</i> , sulcus inframandibularis
<i>f.olf.</i> , fovea olfactoria	<i>s.l.</i> or <i>s.lab.</i> , sulcus labialis
<i>f.pil.</i> , folliculus pili	<i>squ.</i> , squama
<i>gin.</i> , gingiva	<i>s.r.</i> or <i>s.rup.</i> , spina ruptoris
<i>gl.cut.</i> , glandula cutis	<i>v.cer.</i> , ventriculus cerebri
<i>gl.or.</i> , glandula oris	<i>vel.</i> , velum
<i>i.n-h.</i> , invaginatio nasohypophysealis	<i>vil.</i> , villus
<i>l.dent.</i> , lamina dentalis	<i>v.mn.</i> , valvula mandibularis
<i>l.gl.</i> , lamina glandularis	<i>v.mx.</i> , valvula maxillaris
<i>l.i.</i> , labium inferius	<i>v.or.</i> , vestibulum oris
<i>ling.</i> , lingua	<i>v.pal.</i> , valvula palatina
<i>l.lab.</i> , lamina labialis	

PLATE 1

EXPLANATION OF FIGURES

Sagittal sections of the oral region of *Petromyzon* and *Squalus*

6 to 10 *Petromyzon*. Fig. 6, *P. planeri*, 2.7 mm. Harvard Embryological Collection, series 792, section 23. $\times 55$. Fig. 7, *P. planeri*, 4.75 mm. H. E. C., 777, sect. 22. $\times 55$. Fig. 8, *P. fluviatilis*, 27.6 mm. H. E. C., 247, sect. 50. $\times 12$. Fig. 9, *P. fluviatilis*, 42 mm. H. E. C., 250, sect. 66. $\times 12$. Fig. 10, *Petromyzon*, 164 mm. $\times 10$.

11 to 13 *Squalus acanthias*. Fig. 11, 22 mm. H. E. C., 231, sect. 78. $\times 15$. Fig. 12, 50 mm. H. E. C., 444, sect. 143. $\times 30$. Fig. 13, 159 mm. $\times 17$.

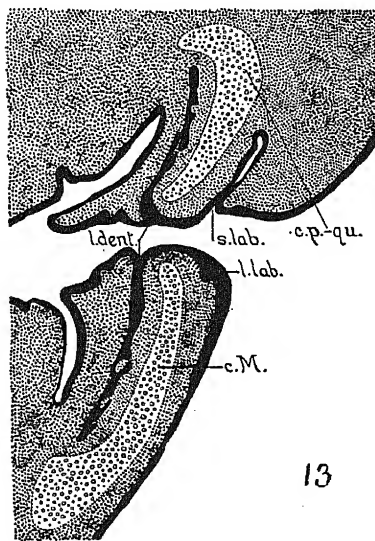
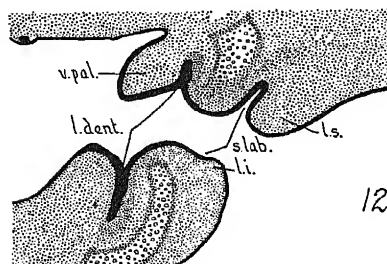
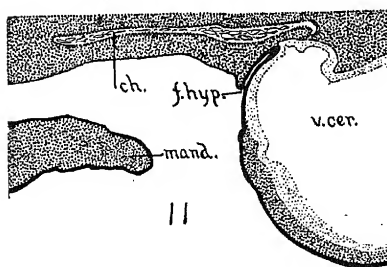
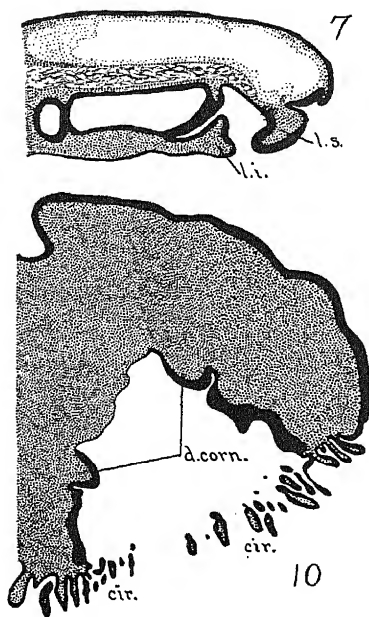
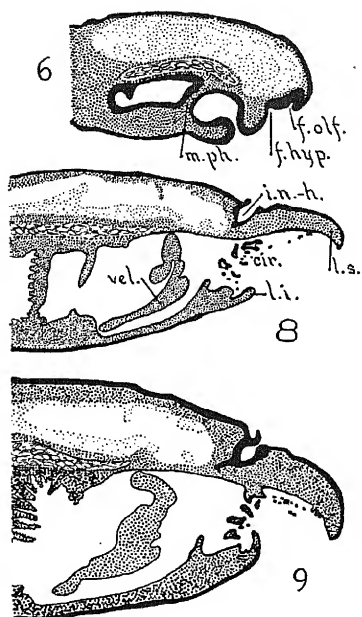


PLATE 2

EXPLANATION OF FIGURES

Sagittal sections of the oral region of fishes

14 *Squalus acanthias*, 520 mm. $\times 7.5$. *a*, lower tooth from crest of the gingival ridge; and *b*, one of the nearest scales from the outer skin of the lower jaw, to show relative size, and direction of spines. $\times 20$.

15 to 17 *Torpedo ocellata*. Fig. 15, 12.8 mm. H. E. C., 689, sect. 68. $\times 15$. Fig. 16, 28.2 mm. H. E. C., 672, sect. 262. $\times 15$. Fig. 17, 51.5 mm. H. E. C., 711, sect. 607. $\times 15$.

18 *Raja erinacea*. 47.7 mm. $\times 15$.

19 *Salvelinus fontinalis*. 25 mm. H. E. C., 581, sect. 85. $\times 30$.

20 and 21 *Opsanus tau*. Fig. 20, 8 mm. H. E. C., 116, sect. 48. $\times 25$. Fig. 21, 41.8 mm. H. E. C., 1177, sect. 423. $\times 25$.

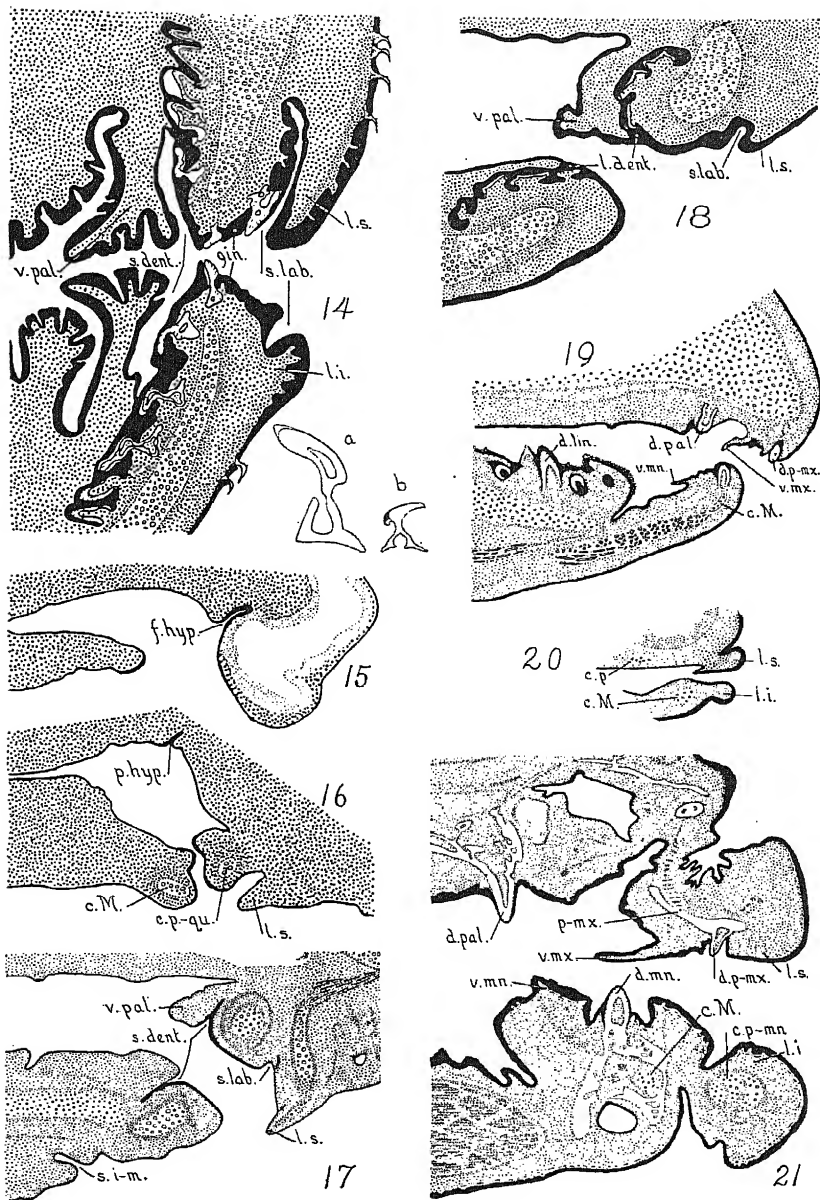


PLATE 3

EXPLANATION OF FIGURES

Mouths of fishes and amphibians

22 and 23 *Gadus morrhua*. Fig. 22, sagittal sections of the lips of an adult codfish, near the median line. $\times 5$. Fig. 23, sketch of the circumoral folds of the adult.

24 and 25 *Amblystoma punctatum*. Fig. 24, 8 mm. H. E. C., 187, sect. 50. $\times 25$. Fig. 25, 26 mm. H. E. C., 655, sect. 145 (with vomeropalatine teeth as in sect. 141). $\times 25$.

26 *Necturus maculatus*. 31.4 mm. H. E. C., 537, sect. 121 (combined, as to teeth, with sections 120 and 122). $\times 25$.

27 *Cryptobranchus allegheniensis*. 350 mm. (adult). $\times 10$.

28 *Spelerpes bilineatus*. 60 mm. (adult). $\times 25$.

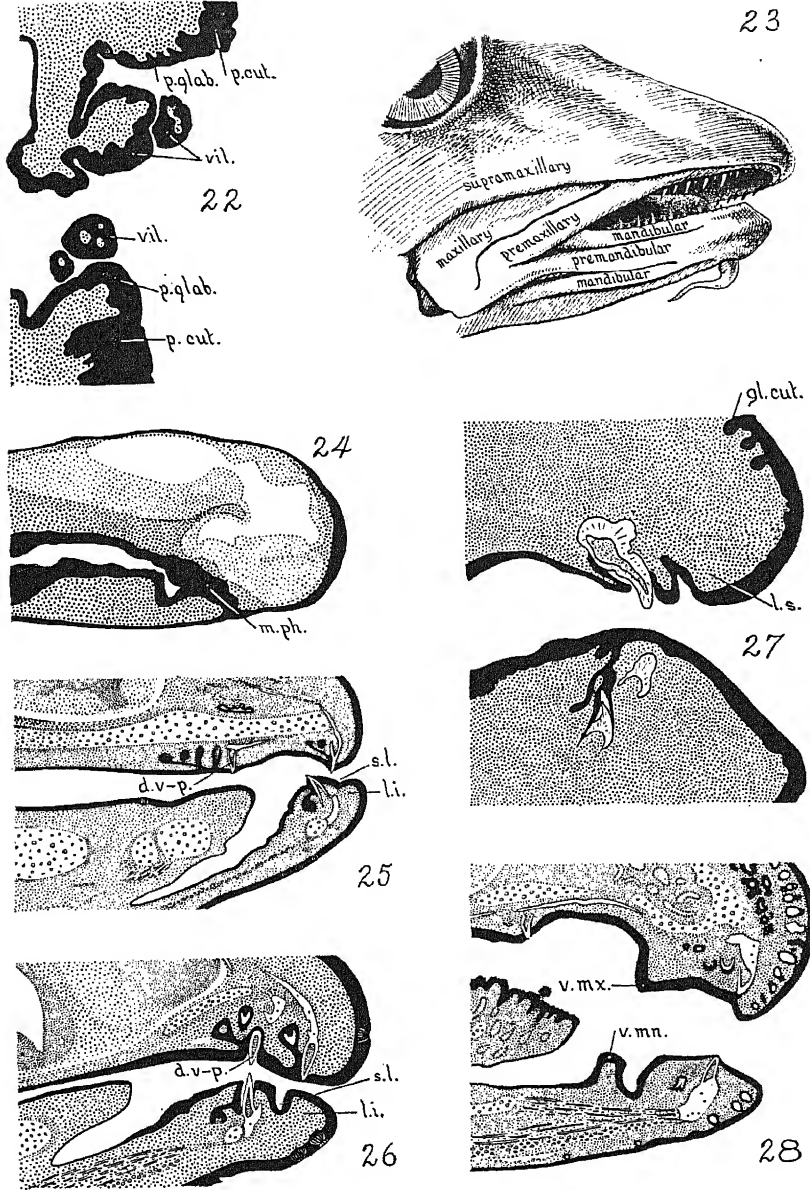


PLATE 4

EXPLANATION OF FIGURES

Mouths of amphibians and reptiles

29 to 31 Sagittal sections of tadpoles of *Bufo lentiginosus americanus*. Fig. 29, 12 mm. H. E. C., 1190. *A*, near the median line, sect. 126; *B*, through the corner of the mouth, sect. 81. $\times 54$. Fig. 30, 22.4 mm. H. E. C., 1153, sect. 144. $\times 35$. Fig. 31, toad of 10 mm. H. E. C., 1135, sect. 167. $\times 32$.

32 *Amblystoma punctatum*. 12 mm. H. E. C., 665. Sagittal sections approaching the corner of the mouth; *A*, sect. 70; *B*, sect. 80. $\times 37$.

33 and 34 *Aristelliger praesignis*. Fig. 33, 7.2 mm. H. E. C., 1629, sect. 91. $\times 40$. Fig. 34, 8.8 mm. H. E. C., 1716, sect. 115. $\times 40$.

35 *Chrysemys marginata*. 27 mm. Transverse section of the mouth. H. E. C., 1096, sect. 219. $\times 21$.

36 *Trionyx ferox*. Lateral half of a transverse section through the mouth of a newly hatched specimen. $\times 15$.

37 *Chrysemys marginata*. 26.4 mm. H. E. C., 1099, sagittal sect. 557. $\times 15$.

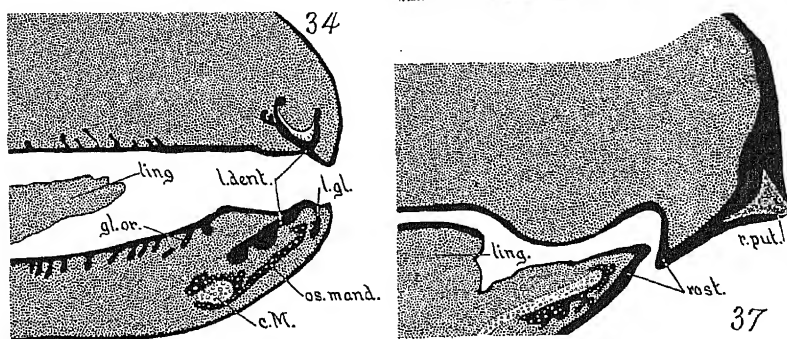
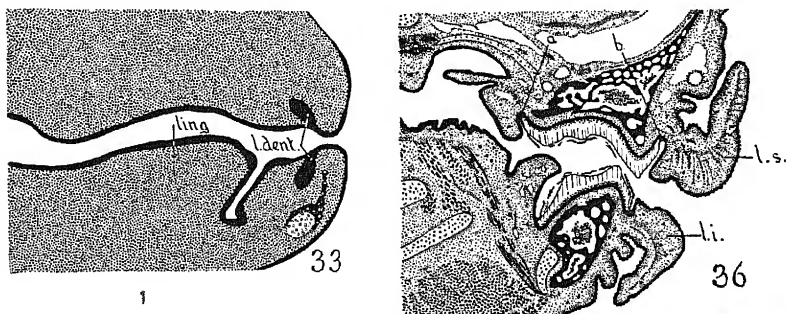
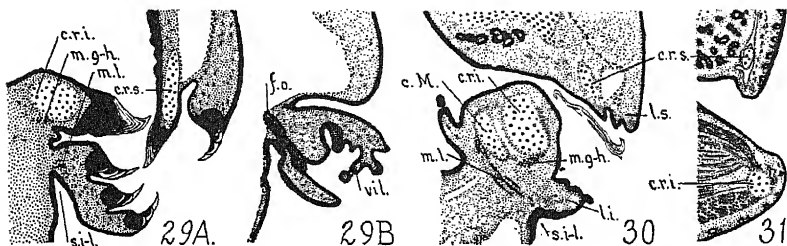


PLATE 5

EXPLANATION OF FIGURES

38 and 39 The beak of chick embryos. Fig. 38, 43 mm. H.E.C., 509, sagittal sect. 356. $\times 15$. Fig. 39, 31 mm. H.E.C., 1967, transverse sect. 1036. $\times 15$.

40 *Typhlops lumbricalis*. Sagittal section of the mouth of an adult 'blind snake.' $\times 40$.

41 to 43 Human embryos, sagittal sections. Fig. 41, 10.2 mm. H.E.C., 736, sect. 147. $\times 20$. Fig. 42, 22 mm. H.E.C., 851, sect. 277. $\times 20$. Fig. 43, 70 mm. $\times 15$.

44 to 47 *Felis domestica*. Figs. 44 and 45, vertical sections of the lower lip of a newborn kitten, 45 being lateral to 44. $\times 12.5$. Figures 46 and 47, vertical sections through the upper lip; 46 from an embryo of 42.5 mm., near the corner of the mouth, $\times 20$; 47 from a newborn kitten, $\times 12.5$.

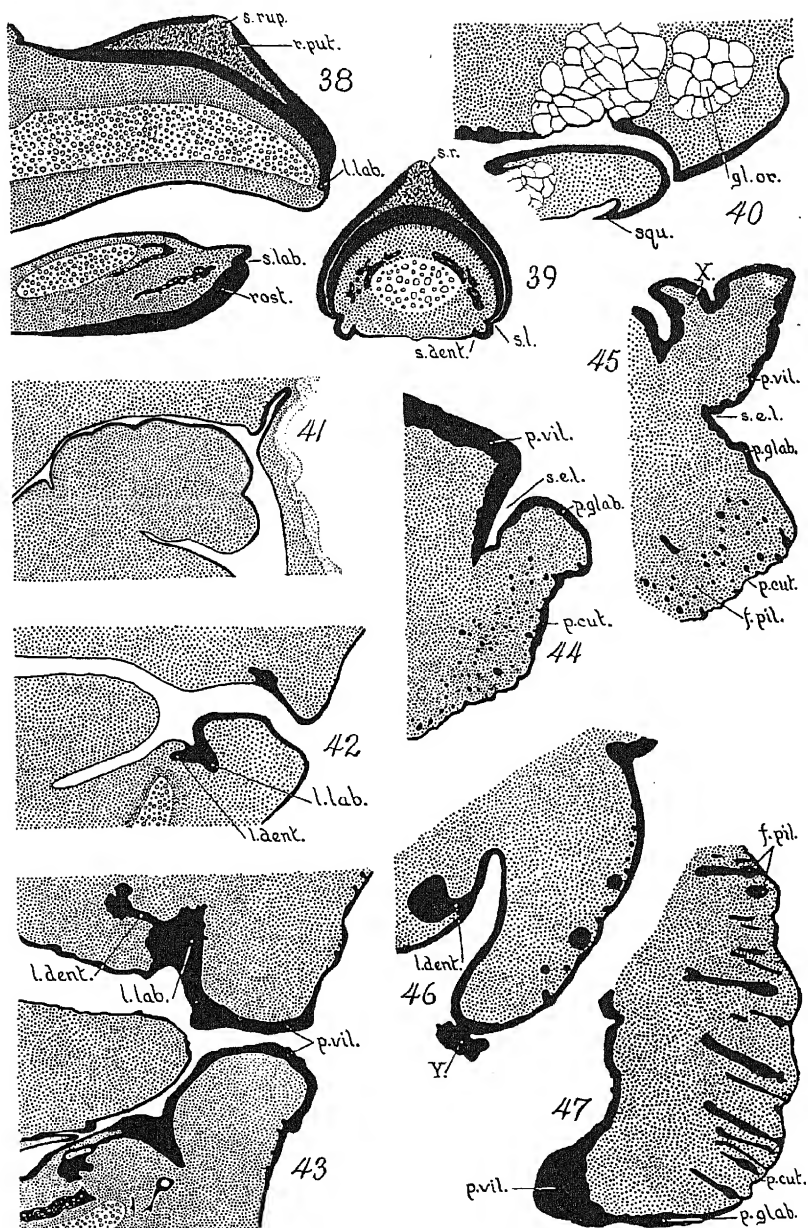


PLATE 6

EXPLANATION OF FIGURES

Mouths and lips of mammals

48 Pig. Vertical section of the lip of a 182-mm. embryo. $\times 7.5$.

49 Calf, at birth. Vertical section through both lips. $\times 7.5$.

50 Rabbit. Vertical section of the lower lip of a newborn rabbit, halfway between the median line and the corner of the mouth.

51 to 53 Guinea-pig. Fig. 51, vertical section of lower lip of a newborn guinea-pig, in front of the right incisor tooth. $\times 15$. Fig. 52, horizontal section through the cheek of an adult, at the angle of the mouth. $\times 5$. Fig. 53, embryo of 18.6 mm. H. E. C., 1787, sagittal sect. 200. $\times 20$.

54 Mouse. Sagittal section of the mouth of a newborn *Mus musculus*. $\times 20$.

55 and 56 Opossum (*Didelphys virginiana*). Fig. 55, sagittal section of the mouth of a 23-mm. specimen, attached to the maternal nipple. H. E. C., 2078, sect. 269. $\times 10$. Fig. 56, vertical section of the lips of a young animal, 170 mm. in length, toward the corner of the mouth. $\times 21$.

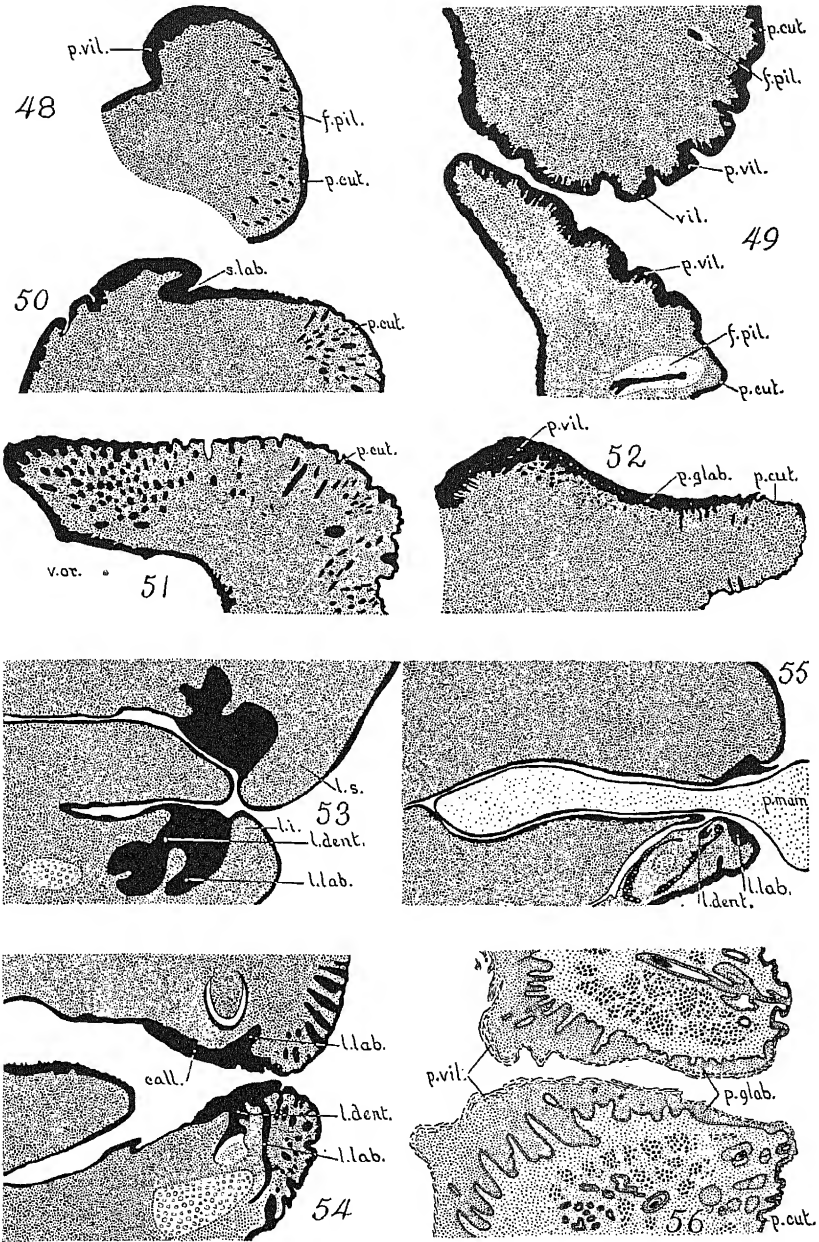


PLATE 7

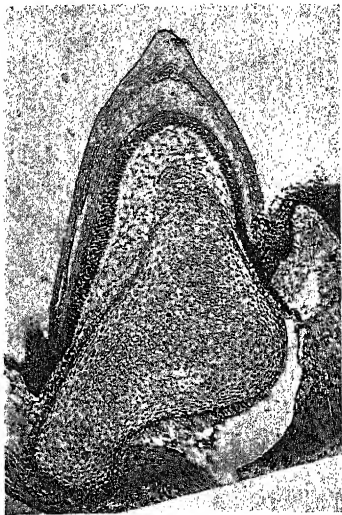
EXPLANATION OF FIGURES

57 *Petromyzon fluviatilis*. Section of a horny tooth, with deep layers representing a replacement tooth, situated above a section of the annular cartilage. From a young specimen (164 mm.). $\times 72$.

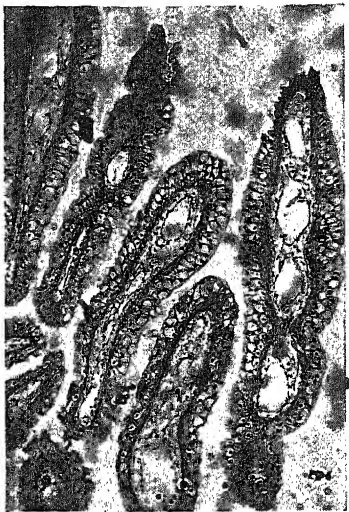
58 *Petromyzon fluviatilis*. Section of oral villi from the dorsal part of the oral funnel. From the same individual as figure 57. $\times 85$.

59 *Squalus acanthias*. Section of a tooth, from an immature specimen of 520 mm. $\times 40$.

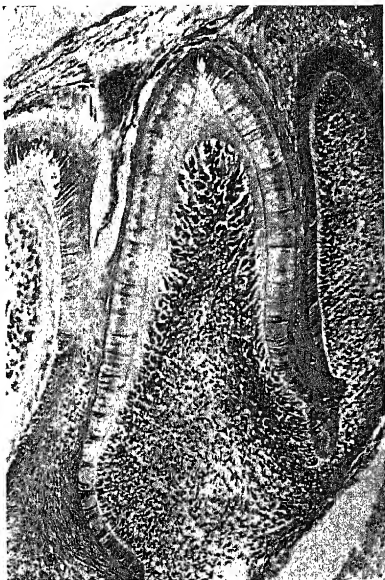
60 *Bufo lentiginosus americanus*. The mouth of a 12-mm. tadpole; the lower jaw shows the horny beak and the three tiers of cornified teeth. H. E. C., 1190, sect. 131. $\times 125$.



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PLATE 8

EXPLANATION OF FIGURES

- 61 *Squalus acanthias*. Ventral aspect of the head: 500-mm. specimen. Slightly reduced.
- 62 *Squalus acanthias*. Mouth, with the upper lip retracted: 500-mm. specimen.
- 63 *Raja ocellata*. Ventral aspect of the head. Two-fifths natural size.
- 64 *Gadus morhua*. Vertical section of a large villus from the upper lip, showing taste buds, with nerves in the connective tissue beneath. $\times 50$.
- 65 *Catostomus duquesnii*. Section of oral plicae of the upper jaw, with taste buds. $\times 44$.



61



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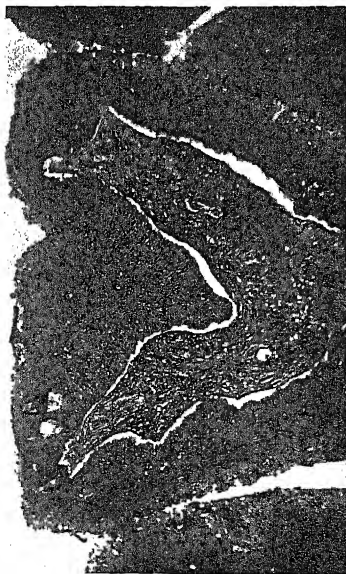


PLATE 9

EXPLANATION OF FIGURES

- 66 Human labial villi. Internal aspect of the lips, being the right half of the mouth of a newborn infant after twenty-four hours in Ringer's solution. $\times 24$. (Photographed by Dr. E. A. Boyden for F. T. Lewis.)
- 67 Vertical section of the upper lip, near the angle of the mouth, from the opposite half of the same mouth shown in figure 66. The fresh tissue was hardened thirty-six hours in Zenker's fluid. $\times 12$. (Preparation and photograph supplied by F. T. Lewis.)
- 68 Sheep. Inner surface of the lips and left cheek of an adult. Large labial and buccal villi. Half natural size.
- 69 Calf. Vertical section of buccal villi of a calf at term. $\times 10$.



66



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69

THE CHROMOSOME CYCLE IN THE ROTIFER *ASPLANCHNA AMPHORA*¹

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The University of Nebraska

FIVE FIGURES

AUTHOR'S ABSTRACT

The diploid number of twenty-six chromosomes was found in the mature parthenogenetic female-producing eggs and also in the somatic cells of the female embryos developing from such eggs. In the maturation stages of a few of these eggs the chromosomes were markedly larger than in the corresponding stages of the majority of the eggs. Whether this size difference of the chromosomes is correlated with male- and female-producing individuals has not been determined. The mature parthenogenetic male-producing eggs contain the haploid number of thirteen chromosomes, and this number was found also in the somatic cells of the young male embryos. The mature sexual eggs contain thirteen chromosomes.

In spermatogenesis the secondary spermatocyte divisions are usually omitted and the secondary spermatocytes develop directly into the motile spermatozoa containing thirteen chromosomes. A few, however, of the secondary spermatocytes divide, forming spermatids containing fewer than thirteen chromosomes. These cells develop into the non-motile and rudimentary spermatozoa.

The motile spermatozoa containing thirteen chromosomes unite with the parthenogenetic male-producing eggs containing thirteen chromosomes, thus producing the fertilized eggs with the diploid number of twenty-six chromosomes. These fertilized eggs develop into female-producing females which reproduce parthenogenetically.

In parthenogenetic reproduction certain female rotifers will produce daughters some of which when mature may produce female offspring, while others may produce male offspring. Shull has found that the kind of offspring produced by these daughters is determined at the time of maturation of the egg from which the daughters themselves are derived. A female-producing mother produces thirty to fifty eggs during her lifetime, and at the maturation of each egg, which later develops into a daughter, the kind of offspring that this daughter will produce is determined. How this is accomplished is unknown.

Shull has studied the maturation stages in the parthenogenetic eggs of *Hydatina senta* and, more recently, Storch and Tauson have studied them in *Asplanchna intermedia* and *Asplanchna priodonta* in an endeavor to discover some clue

¹ Studies from the Zoological Laboratory, The University of Nebraska, no. 159.

to the male- and female-producing mechanism in the chromosome cycle.

In 1920, the author began a study of the maturation stages of *Asplanchna intermedia*. Some of the important stages were found, but owing to the unsatisfactory methods of technique used the work was later abandoned. At this time a very brief summary of this incompleated work was reported, but unfortunately the author misinterpreted some of the stages, and later in another paper misinterpreted some of the stages found by Tauson. However, adopting the embedding technique of Tauson, work was begun on one of the humped rotifers, *Asplanchna amphora*, and considerable success has been achieved.

As the chromosome cycle is very similar to, if not identical with, that of *Asplanchna intermedia*, most of the important features of Tauson's work have been confirmed and also a few additional points of interest have been determined.

MATERIAL AND METHODS

Asplanchna amphora is one of the large ovoviviparous rotifers living in stagnant fresh-water pools during the summer. They can be reared readily in the laboratory in large numbers. Jars holding 40 to 50 liters of rain water were found to be the best adapted for such cultures, to which were added about 20 cc. of dried and split yellow peas every week. These jars were inoculated with pond-water cultures containing miscellaneous protozoa, algae, and other microscopic forms excepting crustacea, and, of course, a few living rotifers. The jars were placed in south and west exposures. Usually, within ten to fifteen days many thousands of females, each containing several eggs and developing embryos, appeared. These were siphoned out and put through a Foerst centrifuge in order to collect them in 5 cc. of water. A trace of the fixing fluid was then added, which somewhat paralyzed the rotifers so that they settled to the bottom of the container.

Several fixing fluids were tried and the most satisfactory one was Flemming's strong solution diluted to about three

parts of the solution to one part of water. Fixation for forty-five to sixty minutes in toto gave excellent results, although in some cases good results were obtained when the fixation period was prolonged one to twelve hours.

The fixed material was placed in running water for twenty-four hours, passed through graded alcohols to 70 per cent, and then bleached by Mayer's chlorine method. Later, it was completely dehydrated and passed into one part absolute alcohol and one part ether for twenty to twenty-four hours; then it was transferred successively to 1 per cent parlodion for two days, 2 per cent parlodion for four days, 4 per cent parlodion for six days, and 6 per cent parlodion for five to fifteen days. Paper boxes suitable in size for sectioning the parlodion block were placed within a jar of ether vapor, and then the material from the 6 per cent parlodion was transferred into them. When the material had settled into a compact mass, the box was transferred to a jar of chloroform vapor fifteen to twenty hours for hardening of the parlodion, and later put into liquid chloroform eight to twelve hours to complete the process of hardening. The whole hardened block was then placed in Apáthy's oil mixture made by weight as follows: Chloroform, four parts; fresh origanum oil, two parts; cedar-wood oil, four parts; absolute alcohol, one part, and carbolic-acid crystals, one part. It was kept in this mixture for five days to several weeks, transferred to benzol for twenty to twenty-four hours, then to one-half paraffin and one-half benzol for four to five hours at 50° to 55°C., and lastly into melted paraffin for four to five hours.

Sections of these masses of rotifers were cut 4 to 5 μ in thickness, and stained in iron-hematoxylin and mounted in Canada balsam. Large numbers of these sections have been made in this manner and examined in an endeavor to find favorable stages. As the chromosomes are numerous, very minute, and usually crowded together, it is a very tedious task to find sufficient stages that are clear enough from which to draw definite conclusions.

Spencer's monobjective binocular microscope equipped with an achromatic condenser, compensating oculars, and a Bausch & Lomb fluorite objective 1.9 mm. and a Leitz 2-mm. apochromatic objective was the most efficient combination obtainable, although many others were tried.

The chromosomes are so small that it was impossible to use a camera lucida in drawing, and, consequently, the size and shape of the chromosomes in the figures are not as accurate as could be desired. In some cases where the chromosomes were crowded the number determined may have been a matter of interpretation, but in other cases where the chromosomes were spread out their number was accurately determined.

THE PARTHENOGENETIC FEMALE-PRODUCING EGG AND THE
FEMALE EMBRYO DEVELOPING FROM IT

Several observers have found that the parthenogenetic female-producing egg of rotifers gives off one polocyte only in the maturation process, and that probably the diploid number of chromosomes remain in the mature egg.

Tauson found in *Asplanchna intermedia* that this diploid number is twenty-four, and Storch found in *Asplanchna priodonta* that it is sixteen. In the present work on the parthenogenetic female egg of *Asplanchna amphora*, a few counts of twenty-six chromosomes were found. Figure 1, A, B, D, shows the maturation spindle containing fifty to fifty-two chromosomes, but these undoubtedly are stages after the chromosomes have split into two parts. They may be late prophases or early metaphases. In an earlier statement these stages were interpreted as prophases showing the diploid number of chromosomes, but this was a misinterpretation. Figure 1, C, E, shows that the diploid number is twenty-six. Figure 1, E, was the only anaphase stage found, and this shows twenty-six chromosomes on the outer end going into the polocyte and twenty-six being left on the inner end in the egg. Figure 1, C, shows a telophase stage containing twenty-six chromosomes on the outer end of the

spindle. Neither second maturation spindles nor second polarocytes were found.

Great numbers of cells in the developing female embryos were examined for favorable stages in which to make chro-

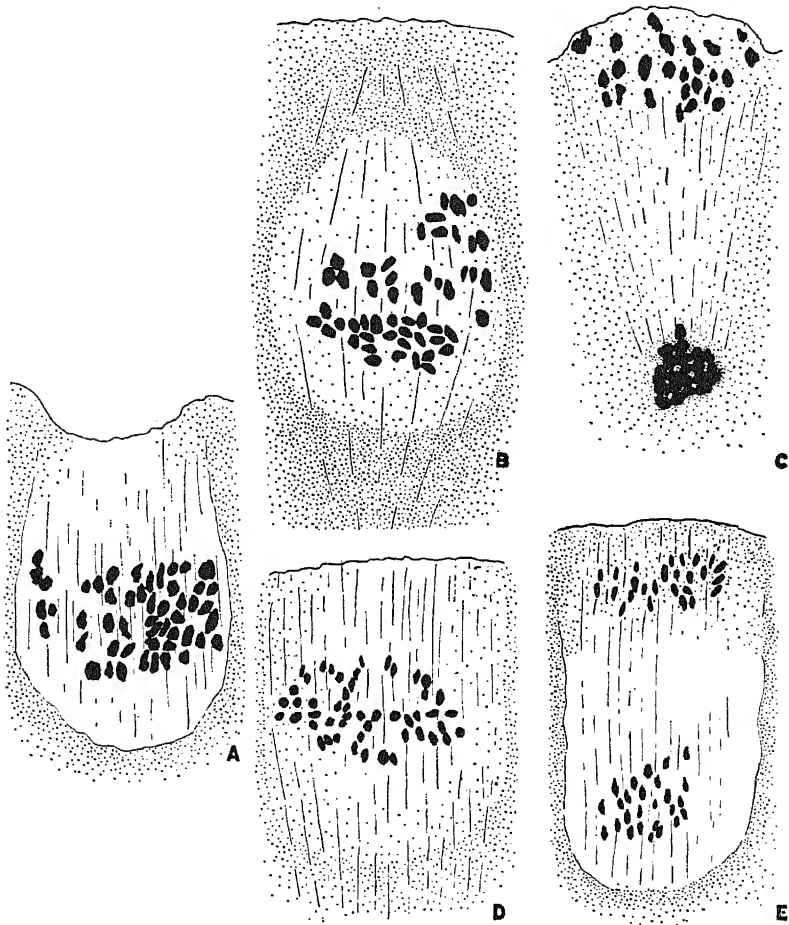


Fig. 1 The parthenogenetic female-producing egg showing the first maturation spindle. A, B, late prophase or early metaphase stages showing fifty and fifty-two large chromosomes; C, telophase showing twenty-six large chromosomes on outer end of spindle; D, late prophase or early metaphase showing fifty small chromosomes; E, anaphase showing twenty-six small chromosomes on each end of spindle.

mosome counts. A few were found in which about fifty to fifty-two chromosomes were counted in the late prophase or early metaphase stages, and a few anaphase stages were seen which showed twenty-five or twenty-six chromosomes toward the ends of the spindles.

Some of these maturing parthenogenetic eggs develop into male- and others into female-producing individuals, and it is of great importance cytologically to determine why one egg develops into a male-producing individual while another develops into a female-producing individual. In some of these maturing eggs (fig. 1, A, B, C) the chromosomes were much larger than in others (fig. 1, D, E). Only a few clear stages of either kind were found in which the chromosomes could be counted, but in many other sections, although unfavorable for counting the chromosomes, nevertheless their relative size could be observed, and it was noticed that in almost all cases the chromosomes were smaller than in figure 1, A, B, C. Whether the size difference in the chromosomes of these two classes of eggs is of significance is a matter for further determination.

THE PARTHENOGENETIC MALE-PRODUCING EGG AND THE MALE EMBRYO DEVELOPING FROM IT

The parthenogenetic male-producing eggs of rotifers produce two polocytes, as has been observed by several workers. Tauson found the diploid number of twenty-four chromosomes in the first polar nucleus, in the first polocyte, and also in the second polar nucleus. On the second polar spindle she found twenty-four chromosomes, but they were so separated that twelve went out into the second polocyte and twelve remained in the nucleus of the matured egg—thus showing that the reduction division takes place in the formation of the second polocyte.

In the present work three prophase stages of the first maturation spindle were found (fig. 2, A to C) in which twenty-six undivided chromosomes were distinctly visible. Here there is no doubt of the count.

No clear first maturation spindles were found, but two first polocytes (fig. 2, D, E) were found, each containing twenty-six chromosomes. A second maturation nucleus (fig.

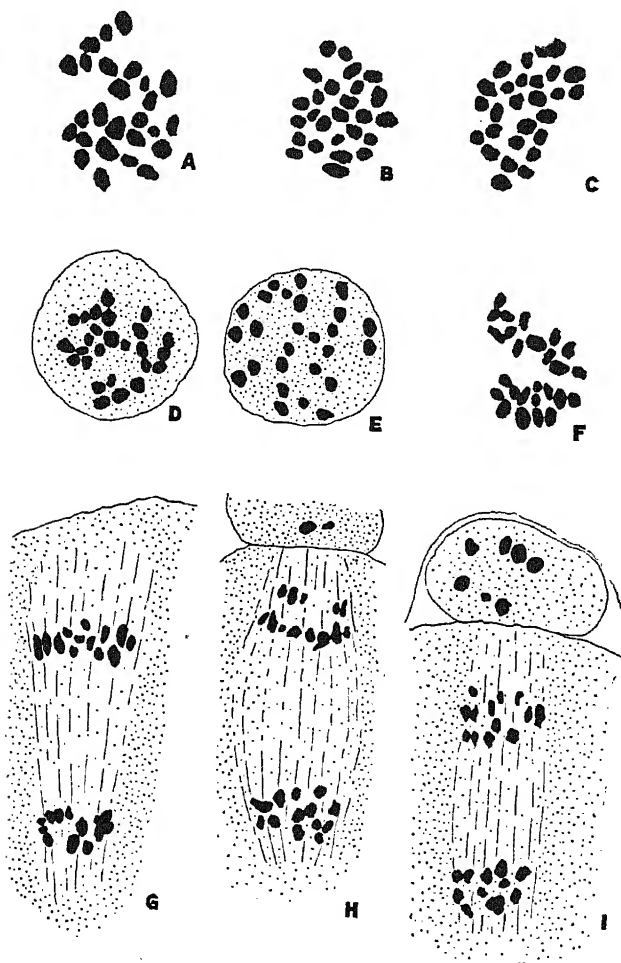


Fig. 2 The parthenogenetic male-producing egg. A to C, prophase stages of the first maturation period, showing twenty-six chromosomes; D, E, first polocytes containing twenty-six chromosomes; F, second maturation prophase showing twenty-six chromosomes; G to I, second-maturation anaphase stages showing thirteen chromosomes on each end of the spindles. H, I also show the first polocyte.

2, F), either in a late prophase or early metaphase, contained twenty-six chromosomes in two groups of thirteen each.

Second maturation spindles (fig. 2, G to I) in anaphase stages have the reduced number of thirteen chromosomes on each end of the spindle, thus agreeing with Tauson that the reduction of chromosomes occurs at the second maturation division.

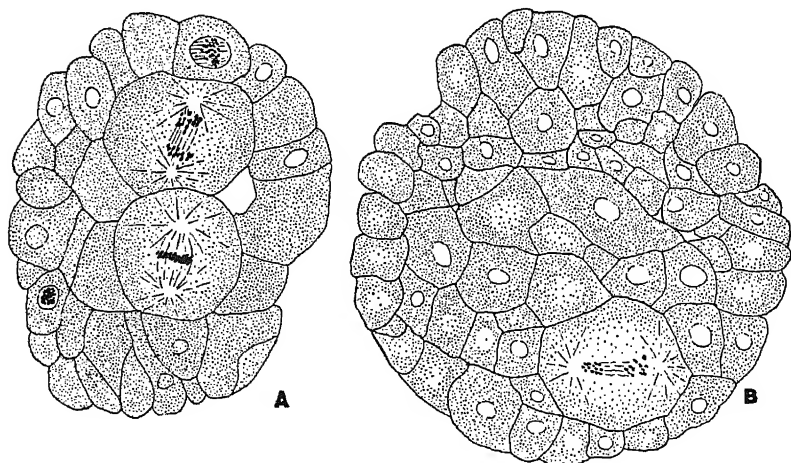


Fig. 3 .Sections of male embryos. A, showing two large endoderm cells in an early embryo in which one is in the anaphase stage with the haploid number of chromosomes on each end of the spindle. Two of the smaller ectoderm cells show the chromosomes after they have divided preparatory to their formation upon the spindle. B, showing a large primordial germ cell in an older embryo in the anaphase stage with the haploid number of chromosomes on each end of the spindle.

Anaphase stages in the early somatic cells of the male embryos (fig. 3, A, B) show thirteen chromosomes in the endoderm cells. Tauson found the diploid number of twenty-four in the early endoderm cells of *Asplanchna intermedia*, but in *Asplanchna amphora* the haploid number of thirteen seems to be present. Although the chromosomes are usually crowded, in general one can readily distinguish an early male embryo from a female embryo by the size of the group of chromosomes in the cells and by the relative number of chromosomes visible in each.

THE SEXUAL EGG

Several observers have determined that the mature parthenogenetic male egg may be fertilized, develop a thick shell, and remain in a quiescent condition for a long period of time. When this fertilized egg eventually develops, a female rotifer is produced. Owing to the different coverings of these eggs and the different appearance of the yolk granules in their cytoplasm, they are very easily identified in sections. Tauson did not publish any observations on these eggs, and, inasmuch as the sections of the eggs of *Asplanchna amphora* were as favorable as those of the other two kinds of eggs, the best ones have been studied. Three very good prophases of the first maturation stages were seen (fig. 4, A to C) in which the chromosome number was clearly twenty-six. In this stage the chromosomes were similar in size to those in the male parthenogenetic egg.

The first maturation spindle (fig. 4, D to F) shows twenty-six diads in the early metaphase and twenty-six single chromosomes on the inner ends of spindles in the anaphase stages. The outer ends show only twenty-one chromosomes, but probably these are imperfect sections and do not show the full number of chromosomes. The first polocyte (fig. 4, G) in the best section found showed twenty-three chromosomes and probably represents the diploid number of twenty-six. The second maturation spindle (fig. 4, G) of this egg, although somewhat imperfect, probably shows the reducing phase with the haploid number of chromosomes separating to go to their respective poles.

Comparing the size of the chromosomes and the maturation stages of these sexual eggs with those of the male parthenogenetic egg, it seems probable that they are identical in their maturation stages, as would be expected.

Wiggenhorn and Whitney have shown that in some of the fertilized eggs of *Asplanchna intermedia* the egg and sperm nuclei do not fuse before the first cleavage stage, and in some embryos of one hundred or more cells the two nuclei may be seen still unfused. Recently, it has been noted that this non-

fusion of the two nuclei is more commonly found when the temperature is excessively high at the time these eggs and embryos are developing. The two nuclei of such an egg were shown in a prophase stage with twenty-six chromosomes in

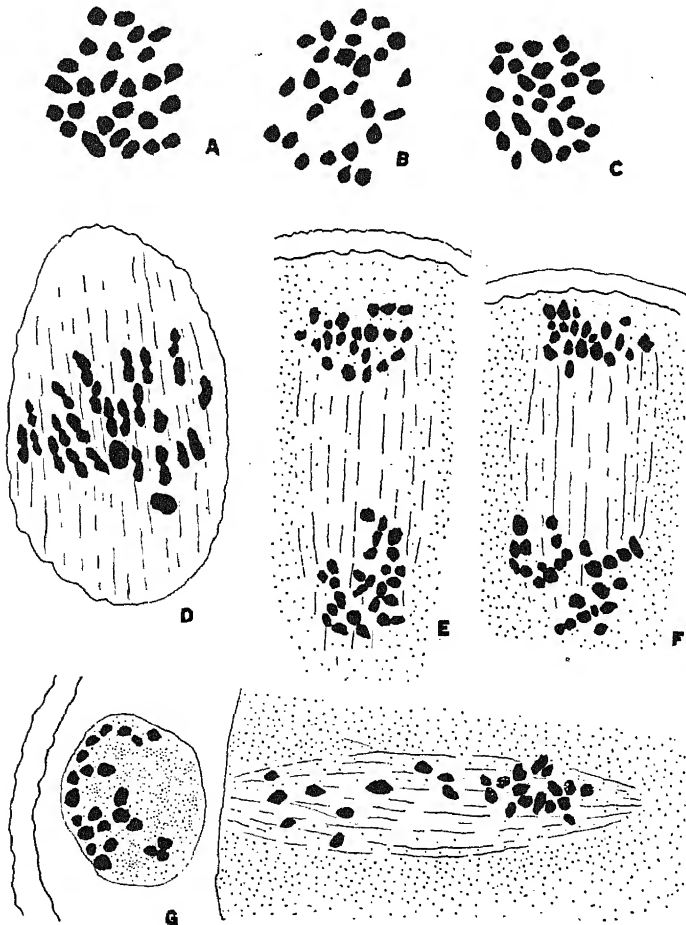


Fig. 4 The sexual egg. A to C, prophase stages of the first maturation period, showing twenty-six undivided chromosomes; D, diad chromosomes going onto the spindle; E, F, first maturation spindle in anaphase stage, showing twenty-six chromosomes on inner end; G, showing first polar body and the second maturation spindle in the anaphase stage with the twenty-six chromosomes separating and moving toward the poles in two groups.

each nucleus. These stages were interpreted as showing the supposed haploid number of twenty-six chromosomes in each nucleus. The number of twenty-six is correct, but instead of their being the haploid number of twenty-six they are the haploid number of thirteen chromosomes which have divided in the prophase stage preparatory to the separation on the spindle.

SPERMATOGENESIS

Owing to the difficulties in the technique and the minuteness and crowded condition of the chromosomes, the stages in spermatogenesis have been difficult to work out. The ovoviviparous forms like *Asplanchna* are more favorable than the oviparous ones in that one can section a whole mother containing several developing male embryos of different ages. All stages of spermatogenesis may be readily found. Although the fixing fluids are favorable and the microscopic accessories have been improved, nevertheless many of the minute details remain undetermined at present.

It has been found previously that two classes of spermatozoa are produced by the males. The majority of the few hundred spermatozoa produced are large with motile tails, but there are always some among them that are smaller and equipped with short and rigid tails. These are probably functionless spermatozoa.

The chromosomes in the spermatogonial stages could not be seen clearly enough to count. Many thousand equatorial plates of the primary spermatocytes were examined, but, owing to the crowded state of the chromosomes, only a few spermatocytes were seen in which the number of chromosomes could be counted (fig. 5, A, B). Twenty-six chromosomes were present. In many other sections eighteen to twenty-five chromosomes were seen.

At first these stages (fig. 5, A, B) were thought to show the haploid number of twenty-six chromosomes, but they really are the thirteen haploid chromosomes which have divided early, forming twenty-six in the prophase stage preparatory to coming onto the spindle. These stages are the equatorial

plates. The primary spermatocytes divide, forming the secondary spermatocytes each containing thirteen chromosomes (fig. 5, C to E). The majority of these secondary

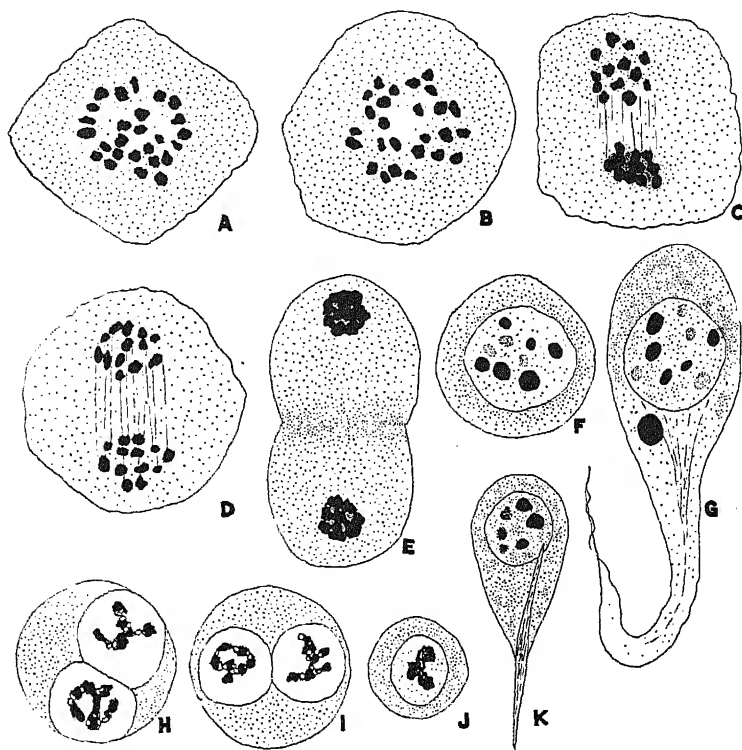


Fig. 5 Spermatogenesis. A, B, equatorial plates of the primary spermatocytes after the chromosomes have divided, forming a total of twenty-six; C, D, late anaphase stages in the division of the primary spermatocytes, showing thirteen chromosomes on each end of the spindle; E, telophase stage of primary spermatocyte; F, secondary spermatocyte which develops into the early motile spermatozoon, G; H, I, secondary spermatocytes containing two nuclei which divide, forming spermatids, J; K, rudimentary and immotile spermatozoon having been developed from the spermatid.

spermatocytes do not divide to form the spermatids, but develop straightway into the motile spermatozoa (fig. 5, F, G) containing the haploid number of chromosomes. Some, however, of the secondary spermatocytes probably do divide to

form spermatids, which in turn develop into the immotile and rudimentary spermatozoa. Three of these secondary spermatocytes were found each containing two nuclei (fig. 5, H and I) which would indicate this assumption, although no mitotic stages were found. The size of the two nuclei in these secondary spermatocytes is about that of the nuclei found in the small spermatids (fig. 5, J) which develop into the rudimentary spermatozoa (fig. 5, K). Furthermore, the size of a whole spermatid cell is about one-half that of a secondary-spermatocyte cell. On this basis of finding a few secondary-spermatocyte cells containing two nuclei, and the relative sizes of these nuclei compared with the nucleus of the small spermatids, together with the relative sizes of these two classes of cells, the assumption is made that these degenerate, immotile spermatozoa are the result of the division of the secondary spermatocytes. The mass of chromatin material in these rudimentary spermatids and spermatozoa seems to be smaller than in the secondary spermatocytes or motile spermatozoa. This probably indicates that the second division of the spermatocytes is a reductional division.

DISCUSSION

The point of greatest interest that stimulated this work on the chromosomes was to determine whether there is any apparent relation between chromosomes and the production of male- and female-producing daughters. By feeding a parthenogenetic mother a scanty diet of green *Chlamydomonas*, her daughters will be female-producing, but if this same mother is fed an abundance of this *Chlamydomonas*, either in the dark or in the light, her daughters will be male-producing. Shull has found that in the presence of an excess of oxygen a mother will produce a higher percentage of male-producing daughters than she would in culture waters having the amount of oxygen that is normally absorbed from the atmosphere. Tauson also has found that a certain excess of oxygen may cause a mother to slightly increase the production of male-producing daughters. She has obtained in some of her

experiments 12.5 per cent higher production of male-producing daughters than in ordinary cultures. Tauson has recently stated that a sudden change in the hydrogen-ion concentration in the water will also bring about a higher production of male-producing daughters.

Shull found that the mechanism which determines the nature of the daughters was in operation in the mother at the time her eggs, that later developed into her daughters, were forming their single polocyte. It occurred to him to study the chromosome cycle in *Hydatina senta* for the clue to this regulating process. He found that the parthenogenetic female-producing egg contained the diploid number of twelve chromosomes, while the mature male-producing egg contained the haploid number of six. He did not study the fertilized egg or the stages in spermatogenesis. He concluded tentatively that the sperm probably has six chromosomes and, when it fertilizes the male-producing egg containing six chromosomes, this fertilized egg develops into a female containing the diploid number of twelve chromosomes in her cells.

Shull working with *Hydatina senta*, Tauson with *Asplanchna intermedia*, and the writer with *Asplanchna amphora* all agree that the mature parthenogenetic male egg has the haploid number of chromosomes and develops without fertilization into a male individual which produces spermatozoa containing also the haploid number of chromosomes. Shull found this number to be six, Tauson, twelve, and the writer, thirteen, in the respective forms studied. Storch has determined in *Asplanchna priodonta* that the mature parthenogenetic male egg has eight chromosomes as the haploid number, but has not determined the number in the spermatozoa. In none of these forms has there been found a marked quantitative difference in the chromosome cycle which would indicate any correlation with male- or female-producing individuals. The only slight difference was found in the parthenogenetic female-producing eggs of *Asplanchna amphora*. In the maturation stages of a few eggs the chromosomes were considerably larger in size than in most of the other eggs.

Whether this larger size indicates lessened metabolism and is correlated with either the male- or female-producing individuals is purely speculative. This size difference is real, but as yet there is no evidence that it is of any significance. Storch has found a difference in the chromosome development and arrangement in the early maturation stages between the parthenogenetic male and female eggs, but has not noted any differences in the early stages of the parthenogenetic female eggs, some of which may develop into male-producers and others into female-producers.

In the young male embryos, Tauson found the diploid number of chromosomes in the endoderm cells and also in the primordial germ cells which are two of the large endoderm cells. In the early division of the latter to form spermatogonial cells a reduction of chromosomes takes place, so that the resulting spermatogonial cells possess the reduced number of twelve chromosomes. This was not verified in *Asplanchna amphora*, inasmuch as one clear anaphase stage of these primordial germ cells was found (fig. 3, B) which showed the haploid number of thirteen chromosomes, contrary to Tauson's observation. The early endoderm cells also showed the haploid number of chromosomes (fig. 3, A) as well as the earlier cleavage cells. How this difference between the males of these two species of *Asplanchna* can be explained is far from clear. Whether the author has failed in the few favorable sections found to correctly identify the endoderm cells that should show diploid chromosomes, or whether the two species differ in this point, which is extremely unlikely, will remain unknown until further work is done on the details of spermatogenesis in these species. Regardless of whether the males of the two species are haploid or diploid in their number of chromosomes in the endoderm cells, both kinds of males produce spermatozoa by a transformation of the secondary haploid spermatocytes.

In the white fly and the cottony-cushion scale insect in which spermatogenesis is quite similar to that of the rotifers,

the Schraders have found that the haploid number of chromosomes is retained throughout all the stages of the male embryo. In spermatogenesis there is one equational division, but no reduction division. These observations are similar to those found in *Asplanchna amphora* by the author. Peacock has found similar haploidy in the spermatogenesis of the males of a certain sawfly.

In the female parthenogenetic egg of *Asplanchna amphora* the division of the chromosomes takes place in the prophase stages of maturation before the spindle is formed, so that when later the chromosomes come upon the spindle the double number appears. This early division of the chromosomes is also found in the primary spermatocyte stages in the young males. However, in the maturation stages of the male parthenogenetic egg and also in the sexual egg, this division of the chromosomes does not take place until the chromosomes are upon the spindle. Storch states that there is a synapsis and formation of tetrads in the early oocytes of these two kinds of eggs in *Asplanchna priodonta* which does not occur in the oocytes of the female parthenogenetic eggs.

In the maturation stages of the male parthenogenetic egg, Tauson seemed to have accepted the diploid number of chromosomes as twenty-four and the haploid number as twelve. In the studies on spermatogenesis ('27) she apparently maintains this conception, although in the anaphase stage (fig. 27) of the last spermatogonial division there are shown thirteen chromosomes going toward each pole and in the anaphase stages (figs. 28, 32), thirteen and twelve chromosomes. Figure 27 would seem to indicate that perhaps this species, *intermedia*, has thirteen chromosomes as the haploid number, as has been found in the species *amphora*.

Storch ('24), although concluding that *Asplanchna priodonta* has sixteen chromosomes as the diploid number, shows about forty-nine chromosomes on the maturation spindle in a late prophase stage (fig. 6) and forty to forty-four chromosomes in a metaphase stage on the first cleavage spindle (fig. 10) of a female parthenogenetic egg.

In a former paper the author counted and classified the two kinds of spermatozoa that could be pressed out of male rotifers. From the few males examined it was concluded that the two classes of spermatozoa were found in the ratio of two motile to one immotile spermatozoa. After examining many thousands of sections of nearly mature male asplanchnae while still inside the bodies of their mothers and in whose sperm sacs both kinds of spermatozoa could be readily seen, the author was impressed with the varying ratios of the two classes of spermatozoa observed. Some males seem to possess only a few rudimentary spermatozoa, while others had about a third as many as of the normal motile spermatozoa. From these recent and more extensive observations the impression is that the ratio of these two kinds of spermatozoa varies considerably in the different male individuals.

SUMMARY

1. The parthenogenetic female-producing egg develops only one polocyte which contains the diploid number of twenty-six chromosomes.

2. In a few maturation stages of these eggs the chromosomes were large, but in most cases they were small. Whether there is any correlation between these eggs showing all larger or all smaller chromosomes and the male-producing or female-producing individuals developing from them is undetermined.

3. The parthenogenetic male-producing eggs develop a first and a second polocyte. The first contains the diploid number of twenty-six chromosomes and the second contains the haploid number of thirteen chromosomes.

4. The ectoderm cells of several male embryos and the early endoderm cells of two male embryos studied contain the haploid number of thirteen chromosomes.

5. The maturation stages of the sexual eggs are identical with those of the parthenogenetic male-producing eggs.

6. The motile spermatozoa containing the haploid number of thirteen chromosomes are formed directly from the second-

ary spermatocytes. The division of the secondary spermatocytes to form spermatids is omitted.

7. A few, however, of the secondary spermatocytes divide, forming spermatids, and these develop into the smaller and rudimentary spermatozoa which contain a smaller amount of chromatin material than the motile spermatozoa.

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THE LEUCOCYTES AND LEUCOCYTOPOIETIC ORGANS OF AN OLIGOCHAETE, PHERETIMA INDICA (HORST)

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FIVE PLATES (FORTY-NINE FIGURES)

AUTHOR'S ABSTRACT

The perivisceral fluid of *Pheretima indica* (Horst) contains five types of leucocytes: lymphocytes, monocytes, granulocytes, lamprocytes, and linocytes. The granulocytes differentiate either from free lymphocytes, from peritoneal epithelial cells lining the leucocytopoietic organs, or from lymphocytes (hemocytoblasts) embedded in these organs. The lymphocyte is a hemocytoblast. The eosinophilic granulocyte is the most numerous of the several types of granulocytes. Morphologically and tinctorially, it resembles the eosinophil of fishes. The eosinophil granule is thought to arise either by a ripening of a basophil granule and to pass through a metachromatic phase during this process, or by being formed immediately without such a ripening process in small hemocytoblasts. The stimulus for the excessive production of eosinophils is thought to be the degree of infection of the leucocytopoietic organs by a species of the gregarine, *Monocystis*.

A series of segmentally arranged leucocytopoietic organs is described for the first time in the oligochaetes. These organs are essentially foldings of the septa and offer sacculations in which leucocytopoiesis may take place.

A discussion of the possible phylogeny of the hemocytopoietic organs of the invertebrates and vertebrates is given.

INTRODUCTION

One of the outstanding problems of hematology is the fate of the lymphocyte. We know from the studies of Maximow, Danchakoff, and others that the lymphocyte arises from embryonic mesenchyme cells which are called hemocytoblasts. But is the lymphocyte a hemoblast per se? Much has been written pro and con regarding the interpretation of the lymphocyte as a hemocytoblast. Maximow ('27), in a critical examination of the accumulated data regarding the developmental potentiality of the stem cells of lymphoid and myeloid tissues in the vertebrates, concludes that most of the morphologic and physiologic evidence favors the view that the hemocytoblasts of myeloid tissue are identical with the lymphocytes of lymphoid tissue. The stem cell does not have the same detailed histologic structure under diverse environmental conditions. In general, it is regarded as a large cell

with a large nucleolated nucleus enclosed in a meager shell of basophilic cytoplasm. This hemocytoblast can, in lymphoid and myeloid tissues, give rise by mitotic division to smaller daughter cells which retain in a latent condition hemocytoblastic potentialities. The small lymphocyte resulting from a series of such divisions is carried throughout the body and, although in a temporary inactive state, it can differentiate into any type of blood cell under suitable environmental conditions. In some regions monocytes arise from this type of cell. In other regions it hypertrophies and becomes a large hemocytoblast or large lymphocyte, returning thereby to the conditions of the stem cell from which it originally arose. In still other regions of the body the small lymphocyte can directly differentiate into a granulocyte. The direction of differentiation of the hemocytoblast seems to be dependent upon the environment provided by the surrounding tissues. In establishing the identity of the hemocytoblast with the lymphocyte, Maximow thinks that such conditions as detailed nuclear structure, cytoplasmic granule content, and peroxidase reaction, which have been used by some investigators to distinguish one from the other, are of little diagnostic value.

If the identity of the lymphocyte with the hemocytoblast can be established in the vertebrates, where there are complex relations between tissues and hemopoietic organs, it would seem that a comparable relationship between hemocytoblast and lymphocyte could be found in the invertebrates, where less complex relationships obtain. In comparison with the mass of data which has been accumulated regarding the leucocytes of vertebrates, that on the conditions of the leucocytes of the invertebrates is very meager. Cuénot ('91), Kollmann ('08), M. Prenant ('22), and Romieu ('23) are the leading contributors to this field of hematology. Considered as a possible starting-point for a unitarian conception of the lymphocyte-hemocytoblast relationship, the investigations of Prenant on the parenchyma of the Platyhelminthes are the best thus far brought forward. In the parenchyma of the Platyhelminthes, which Prenant compares with the embryonal

connective tissue of vertebrates, certain cells are present which resemble morphologically and functionally the hemocytoblasts of the vertebrates. These cells are large rounded cells lying in the intercellular spaces of the parenchyma. They have large basophilic nuclei and thin shells of basophilic cytoplasm. They can fuse with each other or the surrounding parenchyma and evolve into connective-tissue cells, or they can by mitotic division give rise to smaller cells which resemble the lymphocytes. Prenant makes a distinction between lymphocyte and hemocytoblast on the basis of the absence of the chondriome and nucleoli in the former. Consequently, he cautiously states that he cannot be sure that one is identical with the other. Yet, as we have seen from the conditions in the vertebrates, such nuclear and cytoplasmic structures are not of sufficient importance to distinguish one from the other. Furthermore, evidence for the passage from the smaller lymphocyte into the larger hemocytoblast is found in the polyclads, wherein all intermediate stages between small lymphocyte-like cells and hemocytoblasts are seen. Prenant, however, refuses to commit himself on this point, and suggests that the lymphocyte possibly differentiates into a larger hyaline cell, the monocyte. This cell resembles the vertebrate monocyte, but has a peroxydase reaction lacking in the classic monocyte. It does not store the salts of iron, and it has only the phagocytic properties of a microphage.

The importance of Prenant's investigations inheres in the demonstration of a definitive condition of connective-tissue differentiation in the lowest group of tridermic animals which suggests the primitive conditions from which the leucocyte-connective tissue relationship of the vertebrates may have originated during the course of phylogeny. Lymphocyte and hemocytoblast in their lowest terms are identical, and this identity is retained throughout the evolutionary series which leads up to the vertebrates.

According to Prenant, there are no true granulocytes in the Platyhelminthes. There are certain cells in the paren-

chyma which contain granules having an affinity for acid stains. These are the rhabdites and erythrophil bodies. Prenant thinks that these cells cannot be considered as comparable to the eosinophilic granulocytes of the vertebrates, because they are non-amoeboïd and have no peroxydase reaction. Among the triclads, however, there is an evanescent cell which has the nuclear and cytoplasmic characteristics of the hemocytoblast of other flatworms, but which contains eosinophilic and basophilic granules. The latter type of granule is the smaller, and Prenant suggests that it represents a stage in the development of the eosinophilic granule. These cells are very sparse and are found only in young forms. Prenant cannot be sure that they arise from hemocytoblasts, but from their similarity to these cells such a possibility of differentiation seems suggested.

In the nemertines, a class usually grouped with the Platyhelminthes, there are present two or three vascular channels which run the length of the body. These channels are lined with endothelium and enclosed in a thin layer of muscle. Within these vasa Prenant finds two types of cells, lymphocytes and erythrocytes. The lymphocytes are small and the erythrocytes are large. The erythrocytes are thought to contain hemoglobin, but the only evidence for this view is the color change of the fluid under different concentrations of oxygen and the acidophilic tinctorial reaction of the cytoplasm of the cells. These cells are ovoid in shape, and have nuclei which vary in shape and concentration of the chromatin with the age of the cell. In young cells they are deeply chromatic and in old cells, pale. Prenant thinks that the lymphocytes of the vasa are identical with those of the parenchyma and that they enter the vasa by diapedesis. Once in the vascular system, they may possibly differentiate into erythrocytes, since all stages, both morphological and tinctorial, can be found between the lymphocytes on the one hand and the erythrocytes on the other. If this be the case, it serves as a beautiful example of the potentiality of the lymphocyte as a hemocytoblast and would seem to indicate that

its fate is determined by its environment. Such an organization as is found in the nemertines may be regarded as a step in the organization of a definite vascular system with concomitant differentiation of the enclosed cells.

The transition from such an organization as is found in the nemertines to that which obtains in the annelids is very slight. The vascular system in most annelids is very much more complex, and the small-meshed parenchyma is replaced by a series of large serous cavities which lie between the gut and the body wall. In the polychaete branch of this phylum, Romieu ('23) has shown that, despite great differences in the morphologic relationships of the vasa and the serous cavities, the cellular elements differentiate along the lines laid down in the Platyhelminthes. In most members of the polychaete class the vascular cavities are distinct from the serous cavities which Romieu takes to represent the lymphatic spaces of the vertebrates. The vascular system when present usually contains the respiratory pigment in solution. Exceptions to this are found in only one species, *Magelona papillicornis*, in which the pigment in the vascular system is enclosed in anucleate erythroplastids. When vasa are lacking, as in *Glycera*, the pigment is contained in free cells of the perivisceral cavity.

In the polychaetes the principal leucocyte of the serous cavities is the lymphocyte, which is endowed with hemocytoblastic properties in that it can differentiate into either monocytes or granulocytes. In most cases this differentiation takes place as the cells move through the perivisceral cavity, definite leucocytopoietic organs being absent. But in some groups, as in *Glycera*, the peritoneal cells in the region of the nephridia become rounded and resemble the lymphocytes. These cells, which Romieu calls hemocytoblasts, can detach as lymphocytes or can differentiate into erythrocytes and then detach. There is no evidence that the peritoneal hemocytoblasts produce granulocytes while they are in an attached condition. The granulocytes which are present are usually eosinophilic, except in *Glycera*, where large granulocytes

become metachromatic and then basophilic—a change in tinctorial reaction which Romieu thinks is due to age. From the above brief description of the conditions of hematologic differentiation in the polychaetes it would appear that the lymphocyte is a hemocytoblast and that its differentiation depends upon the environmental stimuli.

In the oligochaetous annelids of the family Lumbricidae, whose hematology has been studied chiefly by Cuénot ('91), Rosa ('96), Keng ('95), and Kollmann ('08), lymphocytes, monocytes, and granulocytes have been found. Cuénot and Kollmann regard the lymphocyte as possessing the potentiality of the hemocytoblast of the vertebrates in that it can give rise to the monocyte or to the granulocyte as it floats freely in the perivisceral cavity. But both of these investigators deny the presence of definite leucocytopoietic organs in the Lumbricidae. The conditions in another family of oligochaetes, the Megascolecidae, have been overlooked in this regard. In *Pheretima indica*, which is a member of this family, Beddard ('90), discovered a series of segmental organs which he called the septal sacs and which histologically are composed of peritoneal cells and muscle; the latter continuous with the septum to which the organs are attached. Beddard attached no leucocytopoietic function to these organs. Schneider ('95) studied these same organs in the same species, and concluded that they were phagocytic organs. He noted that the lumina of the septal sacs, which he called lymphoid organs, contained numbers of cells with refractile granules which he interpreted as degenerating chloragocytes. He made no tinctorial study of these cells and hence was misled as to their real nature and significance.

In the course of the present investigation I finally studied *Pheretima indica* after I had sought in vain in other oligochaetes for an organ which could possibly be leucocytopoietic in function. Here I found that the lymphoid organs of Schneider, when studied with the Helly-eosin-azur II hematologic technique, revealed a condition which suggested a leucocytopoietic function. Pursuant of this lead, I have studied in

detail the leucocytes and the leucocytopoietic organs of this particular earthworm. As a result of this investigation, I have found evidence which substantiates the view that the lymphocyte and hemocytoblast are identical and, furthermore, that the cells in the lymphoid organs of Schneider are primarily hemocytoblasts with a granulocytoblastic function—a condition which has not hitherto been found in the oligochaetes and which may be interpreted as representing a possible step in the phylogeny of the leucocytopoietic organs of the vertebrates.

MATERIAL AND METHODS

Pheretima indica (Horst), the oligochaete whose perivisceral fluid forms the basis of the following study, is a member of the family Megascolecidae. The original classification was made by Kinberg ('66), more fully described by Horst ('77) as a perichaete from Java, and finally classified by Michaelsen ('00). *Pheretima indica* has been reported from Florida and Georgia by Michaelsen ('94). It has been found in hothouses in Europe and the United States. The present specimens were collected at Charlottesville, Virginia, in July and September, 1927, and classified as *Pheretima indica* (Horst) following Michaelsen ('00). This is the first record of this oligochaete from Virginia and the farthest north that it has been reported throughout the world. Because of its jumping proclivities, *P. indica* is called the 'eel-worm' by the gardeners of India.

The most prominent external characteristic which distinguishes this worm from such common species as *Helodrilus caliginosus*, *H. foetida*, and *Lumbricus terrestris* is the position of the clitellum extending from segment 14 through 16. The setae vary from twenty-nine on some of the anterior segments to forty-five on some of the posterior ones. They form a complete ring around the segment and make section cutting in this region of the segment very difficult. There are four pairs of spermathecal sacs opening by a series of pores on the intersegmental furrows between the fifth and

sixth, sixth and seventh, seventh and eighth, and eighth and ninth segments, respectively. The spermiducal pores are paired on segment 18. There is a single oviducal pore on the thirteenth segment. Internally, one important specialization is the arrangement and number of the nephridia. Bahl ('20) found 200 to 250 micronephridia per segment. These nephridia open at their proximal ends into a series of tubes which run along the dorsal surface of the intestine and communicate with its lumen by a series of canals. Bahl suggests that this nephridial specialization is an adaptation to prevent loss of fluid. Thus, when the fluid containing solid waste particles enters the intestine, the particles are eliminated with the fecal material, while the fluid is reabsorbed by the intestinal epithelium. The other internal specialization is a series of bilaterally arranged, segmental organs, the leucocytopoietic organs, which are described in detail in this study.

Ordinarily, when *Lumbricus terrestris*, *H. caliginosus*, or *H. foetida* are handled, the perivisceral fluid exudes freely from the dorsal segmental pores, but when *P. indica* is so handled very little fluid escapes, and the body becomes turgid rather than flabby. The fluid itself is very much more resistant to drying than the fluids of the oligochaetes mentioned. The same resistance to drying is characteristic of the cells of the perivisceral fluid. Consequently, much better smear preparations by the drying method can be made from the perivisceral fluid of *P. indica* than from these other worms. Preparations of similar material from *L. terrestris*, *H. caliginosus*, and *H. foetida* were made for comparative study.

The first observations were made of leucocytes in vitro as present in a hanging drop of perivisceral fluid in a moist chamber. For the reactions of the leucocytes to vital stains, neutral red, brilliant cresyl blue, and Janus green were used. The slides used in this method of study were prepared according to Sabin's method ('23). Cells from the perivisceral cavity of worms injected with Chinese ink or carmine in isotonic Ringer's solution were examined to determine the extent of phagocytosis. For the detection of fat, smears of

perivisceral leucocytes were fixed in formalin and stained with a saturated solution of scharlach R in equal parts of 70 per cent alcohol and acetone. Preparations made in this way were mounted in dilute glycerin. Some smears were treated directly with 2 per cent osmic acid to detect fat.

For the study of stained smears of the perivisceral leucocytes and leucocytopoietic organs, Giemsa's and Wright's blood stains were used. Worms fixed in Helly's fluid were sectioned, and stained with eosin-azur II. Finally, gross dissections were made under the binocular microscope for the study of the gross relationships of the leucocytopoietic organs.

OBSERVATIONS

1. The leucocytes free in the perivisceral cavity

Five types of leucocytes are present in the perivisceral cavity of *Pheretima indica*. These leucocytes are in the order of their abundance: *A*) lymphocytes; *B*) monocytes; *C*) granulocytes; *D*) lamprocytes, and *E*) linocytes.

A. The lymphocytes. These leucocytes in vitro are minute spherical cells measuring about 4 to 5 μ in diameter. The nucleus is ovoid and coarsely granular (figs. 26, 27, and 28). The cytoplasm is finely granular and sometimes contains minute vacuoles. This cytoplasm includes an endoplasm enveloping the nucleus and a clear peripheral ectoplasm from which project small filiform pseudopodia. Occasionally, the lymphocyte assumes an elongate shape with the nucleus at one end (fig. 28). In supravital preparations with neutral red, a few granules in the cytoplasm stain rose red. With brilliant cresyl blue, they are violet. They are not affected by Janus green. Often these cells contain chloragogen globules which they have ingested from the perivisceral fluid and which stain red, blue, or green, respectively, with the above supravital stains.

In smears of the perivisceral fluid, stained with either Giemsa's or Wright's stain, the lymphocytes are well preserved and occasionally retain their characteristic pseudo-

podia (figs. 1 and 2). The nucleus is composed of deeply basophilic coarse granules, and there is no trace of a nucleolus. The cytoplasm is more or less deeply basophilic, depending upon the degree of flattening of the cell when smeared. It forms a narrow shell around the nucleus and from its margin project the pseudopodia. Sometimes minute basophilic granules and clear vacuoles are present, but usually the cytoplasm is homogeneous. No mitotic figures were observed in any of the lymphocytes, but a few of them contained double nuclei—a condition which suggests the possibility of amitotic division (fig. 3). Chloragogen globules are present in many of the lymphocytes. These do not arise here, but have been ingested from the perivisceral fluid, which usually contains numbers of free globules.

Lymphocytes observed free in the perivisceral cavity of sections of worms fixed with Helly's fluid and stained with eosin-azur II have the same morphologic characteristics as those in smears. Tinctorially, the nucleus is more lightly basophilic and less densely granular (fig. 5). The cytoplasm is also more lightly basophilic than in the smears.

Lymphocytes from the perivisceral fluid of a worm injected with Chinese ink contain numerous ink particles (fig. 29). In some cells the cytoplasm is so laden with granules that the nuclei, both in the vital and fixed preparations, are obscured.

In both the vital and smear preparations the lymphocytes are frequently found massed together in aggregate plasmodia. In sections, however, there is no such massing of the lymphocytes in the perivisceral cavity. From these facts it is concluded that the plasmodial formation takes place when the diffusion currents set up in drop and smear preparations cause these cells to run together, and that plasmodia do not occur under natural conditions in the perivisceral cavity.

As a result of this study of the lymphocyte, the following brief characterization may be made: The lymphocyte is a minute spherical cell, measuring about 4 to 5 μ in diameter; its nucleus is ovoid, coarsely granular, and basophilic; and its cytoplasm is usually deeply basophilic and homogeneous,

forming a narrow shell around the nucleus. The pseudopodia are filiform and short, usually clumped at one end of the cell. There is a diffuse reaction of the cytoplasm to supravital stains. The cells are highly phagocytic.

B. The monocytes. The monocytes when observed in vitro are apparently of the same nature as the lymphocytes as regards their cytoplasmic content and nuclear structure, but the cells are larger and measure about 7 to 10 μ in diameter, exclusive of the pseudopodia (figs. 30, 31, and 32). The cytoplasm is finely granular and has more irregular granulations than the lymphocyte. There is more cytoplasm around the nucleus than in the lymphocyte. The nucleus is of the same size as that of the lymphocyte, and is ovoid and coarsely granular as well. The pseudopodia differ markedly from those of the lymphocyte in that they are usually broad and petaloid and only occasionally filiform. These pseudopodia frequently change their shape. Figures 30 to 32 show the changes in shape of the pseudopodia of the same cell at intervals of fifteen seconds. These cells often contain yellowish chloragogen globules which they have ingested from the perivisceral fluid.

A typical monocyte in a smear stained with Giemsa's or Wright's stain has certain definite tinctorial reactions which distinguish it from the lymphocyte. In contrast with the lymphocyte, the nucleus is less dense and less basophilic, although coarsely granular (fig. 4). It is sometimes bean-shaped (fig. 7). There are two tinctorially and morphologically distinct zones in the cytoplasm: an inner one tinctorially acidophilic by virtue of a content of minute acidophilic granules, and a peripheral zone which contains a number of irregularly shaped basophilic granules among which are intermingled minute vacuoles (fig. 4). The petaloid pseudopodia are sometimes preserved as flat, plate-like projections with a faint acidophilic tinge (fig. 7). Several monocytes were observed in which there were two nuclei (fig. 8), but none showed any evidence of mitosis. The monocytes form aggregate plasmodia under the same conditions as do the lymphocytes.

Neutral red, brilliant cresyl blue, and Janus green used supravivally have the same effect upon the cytoplasm of the monocytes as they have upon that of the lymphocytes, i.e., coloration of some of the granules of the cytoplasm.

Practically all of the monocytes in the perivisceral fluid from worms injected with Chinese ink contain ink particles (fig. 33). In one cell, in which the process of phagocytosis was in progress, the method by which the ink particles were ingested was observed. Four particles were taken into the cytoplasm during the observations. One particle near the cell was dancing in the surrounding fluid until it came into contact with a pseudopod. As soon as the particle reached the endoplasm, a vacuole was formed and the particle was drawn into the vacuole, which then moved deeper into the cytoplasm.

Monocytes in the perivisceral cavity of worms fixed in Helly's fluid, sectioned, and stained with eosin-azur II very frequently retain their pseudopodia. In such cells the cytoplasm shrinks to such a degree that it is difficult to see it because of the surrounding refractile layer of clear pseudopodia. But in those cells in which the pseudopodia have been withdrawn upon fixation, the nucleus appears to be less granular and less dense than in the smear preparations (fig. 6). The cytoplasm is more acidophilic than that of the lymphocyte in sections, and is usually homogeneous. Thus, the most distinguishing characteristic of the monocyte free in the perivisceral cavity is the presence of petaloid pseudopodia.

In brief, the monocytes are leucocytes characterized by an ovoid, coarsely granular nucleus with a finely granular cytoplasm. Their pseudopodia are petaloid and ever-changing in shape. In Giemsa's and Wright's stains the cytoplasm has two tinctorially different zones, an inner acidophilic zone and a peripheral zone filled with irregular basophilic granules and minute vacuoles. The cells may contain two nuclei. Furthermore, the monocytes are actively phagocytic and amoeboid.

C. The granulocytes. The great majority of the granulocytes of *P. indica* are of the same size as the monocytes, but some of them are as small as the lymphocytes. Between these

two extremes there are all degrees of size. Occasionally, they are 12μ in diameter. In the living condition the cytoplasm of the granulocytes is filled with small, clear spherical granules (fig. 36). These granules are usually of the same size in a given cell, but sometimes larger granules and vacuoles are present. The nucleus is more spherical than that of the lymphocyte, but, like it, is coarsely granular. It is usually obscured by granules. Peripherally, a few blunt pseudopodia may be present, but they are never as numerous nor as large as those of the monocytes. The cell is continually changing its shape as it moves through the field of the microscope.

In smears stained with Giemsa's or Wright's stain, the granulocytes are all of one type as regards their tinctorial reaction. The granules are spherical in shape and eosinophilic in staining reaction. When the cell is smeared very much, the individual granules are distinct from each other and have a homogeneous rose-red color in most cases (figs. 9 and 11). In these cells the nucleus may lie deeply within the cytoplasm. Tinctorially, it is more bluish than that of the lymphocyte, but is just as granular. No nucleolus is present. In the granulocytes which have not been spread out when the smear was made, the granules are closely massed together and the nucleus is peripheral (fig. 13). This is probably the more normal position. A few cells contain granules which are more purplish red than the above (fig. 20). These I have interpreted as metachromatic granulocytes—a stage in the development of the eosinophils. The explanation of the changes leading to the formation of the eosinophilic granulocyte is best described, however, when the leucocytopoietic organs are considered.

Granulocytes in the perivisceral fluid of worms fixed with Helly's fluid and stained with eosin-azur II show a higher degree of metachromaticity of the granules than is evident in the smears. The vast majority of the granulocytes have granules which are distinctly eosinophilic and have a rose-red tinctorial reaction (figs. 10, 12, and 15). In most of these cells the periphery of the granule alone stains, the remainder being

clear (figs. 10 and 12). This condition is probably caused by the fixing fluid, which makes the tinctorial substance coagulate and condense on the periphery of the granule. Other granulocytes contain granules which have a homogeneous red tinctorial reaction (fig. 15). The granules of these cells are apparently not modified by fixation. The nucleus has the same characteristics as in the smears, but it is not as coarsely granular nor as deeply basophilic. Occasionally, a granulocyte with two nuclei is seen (fig. 14).

Sometimes, granulocytes are present in the perivisceral fluid, intermingled with the other types of cells, in which the granules are bluish purple (fig. 21). These granules are as small as the eosinophilic granules of the homogeneous type, and, like them, are homogeneous and stain throughout their whole extent. The granules are so dense that the nucleus cannot be seen, but its presence is indicated by a light area beneath the granules. These cells I call ripening eosinophils for reasons best explained later in the paper, when the cellular contents of the leucocytopoietic organs are described.

No ink particles were observed in the granulocytes from worms injected with Chinese ink—from which it is concluded that these cells are non-phagocytic.

In supravital preparations with neutral red, the granules of the granulocytes have a yellowish-brown tinctorial reaction. I interpret this reaction as indicative of the alkaline nature of the contents of the granules. With brilliant cresyl blue the tinctorial reaction is violet. Janus green does not stain the granules.

In brief, the granulocytes from the perivisceral fluid are leucocytes with small spherical granules, alkaline in nature, and mostly eosinophilic. Some granulocytes contain granules with a metachromatic purple tinctorial reaction which I interpret as stages in the development of eosinophilic granules. The cells may have pseudopodia of the petaloid variety. The granulocytes are non-phagocytic and measure from 4 to 12 μ in diameter. The nucleus is usually spherical and coarsely granular. The presence of two nuclei in some of the granulocytes suggests the possibility of amitotic division.

D. The lamprocytes. The lamprocytes are the largest cells in the perivisceral fluid, excepting detached chloragocytes. They may have a diameter of $22\ \mu$. The lamprocyte has a very distinct cell membrane and does not form pseudopodia. The cell is flat and disc-shaped, usually having an irregular contour. The cytoplasm is filled with a number of closely packed yellowish globules (fig. 41). The nucleus is small and spherical or ovoid, and is usually hidden by the globules.

In supravital preparations with neutral red, the globules stain a light red.

In order to compare the nature of the content of the globules of the lamprocytes with those of the chloragocytes and those of known eleocytes, the latter from *Helodrilus foetida*, these elements were treated with scharlach R. Under this treatment the globules of the lamprocytes were not affected; the globules of the chloragocytes of *Pheretima*, brownish yellow before treatment, became red brown—a reaction indicating the presence of fat. The globules of the eleocytes of *H. foetida*, slightly yellow before treatment, became crescent-shaped and stained red, thus indicating the presence of fat. The chloragocytes of *H. foetida*, with brownish-yellow globules before treatment, showed diverse color reactions in these globules. Some remained brownish yellow and others stained varying shades of red, thus indicating the presence of fat in some of the granules. The globules of the chloragocytes of *Lumbricus terrestris*, used as an additional check on these results, stained a deep red. From these facts it is evident that the content of the globules is not a fat and is not identical with the content of the chloragogen globule. These results are in accord with the original descriptions of the lamprocyte in *Octochaetus multiporus* (Benham, '01) and of the eleocyte in *H. foetida* (Rosa, '96).

In smears stained with Wright's or Giemsa's stain the globules of the lamprocytes have a faintly metachromatic tinctorial reaction (fig. 16). Some of the globules shrink under this fixation and appear as small clear circles. The nucleus is small and pycnotic. These characteristics are quite in con-

trast with the tinctorial reactions of the globules and nucleus of the chloragocyte. In this type of cell the globules have a bluish-green translucent coloration which is sometimes enclosed in a delicate crescent of acidophilic nature (fig. 42). The nucleus is large, vesicular, and has a distinct nucleolus.

In the perivisceral cavity of worms fixed with Helly's fluid, sectioned, and stained with eosin-azur II, the lamprocytes are well preserved. The globules have each a small polar cap at one end which is not stained (fig. 17). Usually, this cap is enclosed in a band of greenish material, which is in turn enclosed in a crescentic area which has a very faint acidophilic tinctorial reaction. In the globules of some lamprocytes the non-tinctorial region is large and the bluish-green crescent is pushed to the side of the globule, thereby eliminating the acidophilic region. In still other lamprocytes the globules are shrunk and only a few are present, while the cell itself is very much wrinkled (figs. 38 and 39). Judging from the series of different stages observed, it appears that the lamprocytes undergo degeneration in the perivisceral cavity. The nucleus in a cell fixed and stained by the above method is a small spherical body, which has not the pycnotic character seen in smears. The globules of the chloragocytes, in contrast to those of the lamprocytes, have in this method a homogeneous light-green tinctorial reaction and the nuclei are large and vesicular.

Lamprocytes from the perivisceral fluid of worms injected with Chinese ink do not contain ink particles.

The lamprocytes may be described briefly as large leucocytes with a content of large, yellowish globules. The content of the globules is not fat. In sections the globule has three different tinctorial zones, the significance of which has not been determined. The nucleus is small and spherical. There is no indication of phagocytosis nor of division stages. The lamprocytes apparently degenerate in the perivisceral cavity. There is no genetic relationship between the chloragocytes and the lamprocytes.

E. The linocytes. The linocytes are cells of about the size of the lamprocytes, but may be larger or smaller, depending upon the stage of development. They differ from all other leucocytes in having the cytoplasm filled with either a single large vacuole (fig. 43), a number of irregularly sized vacuoles (fig. 46), or vacuoles and coiled threads (fig. 44). In the smaller linocytes there may be a single large vacuole which has pushed the nucleus to one side (fig. 43). The nucleus is spherical and granular. The cells are very elastic and, when compressed by movements of neighboring cells, they return to their original shape after the pressure is released. In some of the larger linocytes the vacuoles are obscured by the presence of a coiled thread-like mass which is very refractile and shiny (fig. 44). In still another condition the thread-like mass is broken and pushed through the cell membrane in a series of flaps and knobs (figs. 34 and 35). In several of the linocytes I observed large petaloid flaps, but, since these did not change in shape, I am of the opinion that they are not petaloid pseudopodia. In neutral-red preparations the clear cytoplasm between the vacuoles stains a faint red; the vacuoles are not stained (fig. 46).

In smears stained with Wright's or Giemsa's stain the linocytes contain large irregularly shaped vacuoles between which there is a framework of faintly basophilic cytoplasm (fig. 19). The nucleus is spherical, granular, and basophilic. In smears fixed with 2 per cent osmic acid the thread-like mass observed in the living linocyte shrinks and stains black. From this reaction there is a possibility that the thread-like mass represents the Golgi apparatus of the cell. Such a condition would imply that these cells have a secretory function.

F. Other cells free in the perivisceral fluid. In addition to the leucocytes, the perivisceral fluid contains detached chloragocytes and parietal peritoneal cells. The chloragocytes are easily distinguished from the leucocytes by their large size and by their content of brownish-yellow globules in living preparations. In preparations treated with scharlach R the presence of fat is revealed in the globules. In stained prepa-

rations the globules are yellowish- or bluish-green in tinctorial reaction (fig. 42). The nucleus is large and vesicular with a distinct nucleolus. The chloragocytes have no genetic relationships with the leucocytes. Topographically, they cover all of the larger vasa of the perivisceral cavity and enclose the peri-intestinal blood sinus.

The parietal peritoneal cells also have a content which enables one to identify them readily. In vital preparations they contain a number of short bacteriform rodlets and small vacuoles. The nucleus is ovoid and granular. The rodlets are stained yellowish-brown with neutral red, and violet with brilliant cresyl blue. In smears stained with Wright's stain the rodlets have a metachromatic purplish color (fig. 18). In sections fixed with Helly's fluid and stained with eosin-azur II, the rodlets have a purplish metachromatic tinctorial reaction, while the vacuoles are deeply basophilic and of various sizes. In passing, I may say that Buchner ('21) regards such rodlets in the cells of the other invertebrates as symbiotic plant-like organisms. Trojan ('19), on the other hand, contends that the rodlets of the oligochaetes are mitochondria. My observations support Buchner's rather than Trojan's view in that these structures withstand conditions of fixation which destroy mitochondria.

2. *The leucocytopoietic organs*

The leucocytopoietic organs of *P. indica* are a series of segmental cellular masses developed within the folds of the median dorsal portions of the septa from the twenty-fourth segment to the caudal end of the worm. These masses were first observed by Beddard ('90) in *Perichaeta indica*, the taxonomic name of *Pheretima indica* at that time. He says:

Perichaeta indica is furnished with a series of curious glandular looking bodies in most of the posterior segments of the body; these are attached, close to the middle line on either side of the dorsal vessel, to the posterior side of the septa. They were perfectly recognizable both in transverse and longitudinal sections, though naturally their relations to the septum were better shown by the latter, their

position with reference to the dorsal vascular trunk by the former series of sections.

Structurally these small white bodies consist of a mass of cells continuous with the peritoneal epithelium and probably formed by a local proliferation of its cells; in the interior of each were a few muscle fibers; there was no trace whatever of a central cavity, which occurs in corresponding bodies of the allied genus *Acanthodrilus*. These septal glands were in *Perichaeta indica* solid throughout.

G. Schneider ('95) again studied these organs in *P. indica* with a view to determining their function. After injecting specimens with carmine in water, he found that upon opening a worm from the dorsal surface there appeared a series of segmental organs colored red by the absorbed carmine. These were present from segment 26 to the caudal end and lay in the posterior part of the segment on either side of and above the dorsal vessel. He describes each as an ellipsoidal, flattened organ attached to the anterior face of the septum and connected with its fellow of the opposite side by a strand of phagocytic tissue across the dorsal vessel. Following this gross study, he examined these organs histologically, but not with differential stains which would affect the cytoplasmic content of the constituent cells.

In transverse sections of young worms he found that the dorsal vessel lies in a muscular sheath from the lateral surface of which the lymph glands, as he named them, extend. This sheath is continuous posteriorly with the septum, and the muscle fibers of the septum extend from it into the glands, forming a branching muscular tree, the stroma of the glands. The adjacent segments communicate with each other through the septum at the region of attachment of the glands. According to Schneider, the gland is not a solid mass, as Beddard stated, but a tree-formed branching mass, whose twigs in older specimens lie so close together that the whole gives the impression of a cellular mass pierced by numerous lacunae. The muscle fibers forming the stroma of the gland are covered with thickened peritoneal cells. Within the gland, Schneider observed cells filled with minute strongly refractile granules, such as he saw moving in the perivisceral cavity

without showing any indication of phagocytosis. These cells either lie free in the lumen of the gland or in clumps covered by phagocytic leucocytes. Schneider concluded that the cells with granules were chloragocytes which had dropped into the body cavity, undergone degeneration, and were swept into the lumina of the lymphoid glands by movements of the body. The cells of the lymphoid organs, other than the so-called dead chloragocytes, ingested such dyes as iron-ammonium carmine and indigo carmine, which were introduced into the body cavity in water. Because of the concentration of the dyes in these cells, as well as the presence of dead chloragocytes, Schneider concluded that the lymphoid organs were the important phagocytic organs of the body. Their function was to remove foreign particles from the lymph of the perivisceral cavity as well as to digest and destroy dead chloragocytes and tissue débris which were swept into the lumina of these organs by contractions of the body musculature. Because he could find no evidence of cellular proliferation, this investigator left open the question as to whether or not the lymphoid organs, as he termed them, were leucocytopoietic as well as phagocytic. Kollmann ('08) calls attention to Schneider's work, but dismisses his results as without significance.

The present study of these organs in *Pheretima indica* was undertaken before access was had to Schneider's memoir. With the gross histological characteristics of the lymphoid organs as he described them, I agree, but I differ from him as regards detailed histological characteristics and functional interpretations. I find, as Schneider did, that the organs, which he described as lymphoid organs, are segmentally arranged (fig. 40, *L.O.*). But in the specimens which I examined grossly I found that the organs begin as bilobed elements in segment 24 and are present in all of the more posterior segments. In this region of the body each lobe projects from its junction with the septum anteriorly and lies lateral to the dorsal vessel. Each lobe is connected with its fellow of the opposite side by a narrow strip of tissue similar to that comprising the lobes (fig. 40). In the segments of the posterior

part of the body, where these organs are best developed, each lobe is a racemose structure, whitish in color as contrasted with the dark color of the dorsal vessel and the brownish tint of the intestine. The average length of each lobe in this region is 0.25 mm.; its width, 0.4 mm.; and its depth, 0.6 mm.

Each leucocytopoietic organ is in essence a folding of the septum to which it is attached, but the simplest condition which such a folding could take is never found in the fully developed organ. Since this organ arises from the septum and is continuous with it, it necessarily is a muscular membrane covered on both sides by peritoneal epithelium. As has already been pointed out, the peritoneal epithelial cells have a content of rods and basophilic globules. An epithelium of such constitution is present on both sides of the septum up to the point where the organ begins (fig. 48). The epithelium lining the organ is, however, greatly modified. The lumen of the organ communicates with the perivisceral cavity at the region of the junction of the organ with the septum. The epithelium lining the lumen of the organ is composed of either unmodified, flattened, pavement cells or of eosinophilic granulocytes. The shapes of the cells may vary with the degree of contraction of the muscular layer which this epithelium covers (figs. 48 and 49). A series of lacunae formed by the foldings of the wall of the organ opens into the central lumen. In the deep ends of the lacunae there are thickened cellular cords which are covered peripherally by the outer peritoneal epithelium of the organ. No muscular tissue intervenes between the cellular cords and the epithelial layer in this region (fig. 49). Thus, the characteristic picture of the leucocytopoietic organ is a composite of muscle tissue intermingled with which there is a series of lacunae and solid cords of cells. The cords are the regions where leucocytopoiesis takes place.

In these cords there are present all of the types of leucocytes described as occurring in the perivisceral cavity, with the exception of the linocytes. Granulocytes are the most numerous cells here. One of the most prominent types of granulocytes found in the deeper ends of the cords is that

which contains small granules which have a lightly basophilic tinctorial reaction (fig. 25, *Bo.*). Other granulocytes contain, in addition to these basophilic granules, a few scattered purplish metachromatic granules (fig. 22 and fig. 25, *Eo.m.*). Still others contain mostly purplish metachromatic granules (fig. 23). From cells with the purplish granules a series of stages can be followed which culminates in a type of cell with the brighter-red eosinophilic granules (fig. 24). As a result of these observations, I am led to conclude that the cells with the basophilic granules are immature eosinophils and that the cells with purplish metachromatic granules are ripening eosinophils. The degree of metachromaticity of the granule is an indication of its degree of maturity. Another fact which leads me to this conclusion is the usual absence from the perivisceral cavity proper of cells with lightly basophilic granules. Occasionally, cells with metachromatic granules are found free in the perivisceral cavity (fig. 20), while eosinophilic leucocytes abound. Furthermore, the lacunae leading to the perivisceral cavity from the leucocytopoietic cords are usually filled with eosinophilic granulocytes—a condition which suggests that they are cells which, having matured in the leucocytopoietic organs, are on their way to the perivisceral cavity (fig. 48). Chloragocytes which have a content of green granules are only occasionally observed in the leucocytopoietic organs. Hence, from my material I do not agree with Schneider that the lacunae of these organs are filled with dead chloragocytes. Schneider could not demonstrate the tinctorial differences between the several types of leucocytes and the chloragocytes, because differential staining had not come into use at the time he published his memoir.

Cells resembling lymphocytes are present in the leucocytopoietic cords (fig. 25, *L.*). These cells appear to be the stem cells from which the granulocytes differentiate. There are the possibilities that they have either arisen in situ from the peritoneal epithelium or that they have entered the lumen of the organ from the perivisceral cavity. Evidence for their immediate origin within the organs inheres in their relations

to the epithelium covering the cords and lining the lumina. Occasionally, certain of the covering cells, which are ordinarily of the squamous type, are rounded and project into the cords in such a manner as to suggest a subsequent detachment and association with the cells of the cords. Furthermore, certain of the cells lining the lumina of the organs have a content of eosinophilic granules. These cells are in varying degrees of detachment from the lumen (fig. 48). On the other hand, lymphocytes can be seen in the lumina of the organs. These can be interpreted as lymphocytes entering the organ or leaving it. If they are entering, it is suggested that they are moving to take up a sessile position in the leucocytopoietic cords to function as hemocytoblasts. From these facts it is concluded that the hemocytoblasts which give rise to the granulocytes in the leucocytopoietic organs are either resting lymphocytes or detached peritoneal cells.

In addition to the granulocytes and hemocytoblasts, lamprocytes are also found in the leucocytopoietic cords. They are of all sizes and usually seem to be more degenerate than those which are found free in the perivisceral cavity (fig. 49). The globules of these cells have a very light tinctorial reaction and there is a crescentic basophilic area on one side of the globule. Most of the lamprocytes observed are in the deep ends of the leucocytopoietic cords (fig. 49). From their positions it would appear that they have entered the organs from the perivisceral cavity and have been forced to a deep position by the amoeboid activity of the other cells. In this position they possibly undergo degeneration, and the products of lysis are removed by the lymphocytes.

The leucocytopoietic organs usually contain large numbers of developmental stages of a species of the gregarine *Monocystis*. Frequently the mature stage is present. In other species of oligochaetes the developmental stages of this parasitic protozoan occur in the sperm sacs. Since parasitic infections in other Metazoa result in the development of a greater number of eosinophilic granulocytes than is usual, it is suggested that the high incidence of eosinophils in the leucocyto-

poietic organs of *Pheretima indica* is caused by the presence of these parasites.

Sections of the leucocytopoietic organs were examined for evidences of mitosis. No mitotic figures were observed, but in many of the leucocytopoietic cords the nuclei of the hemocytoblasts were either polymorphic or double (fig. 47). Since polymorphic nuclei are rare in the leucocytes of the perivisceral cavity, it is suggested that these changes in shape are evidences of preparation for amitotic division. This view is further substantiated by the presence in neighboring cells of two nuclei. It is realized that the presence of two nuclei in a single cell may be interpreted as an end product of mitosis as well as amitosis. Consequently, the interpretation of amitosis as the causative factor producing the two nuclei rests upon the observation of polymorphic nuclei in the neighboring cells and the complete absence of any mitotic figures in any of the sections studied.

DISCUSSION

The results of the present study, when compared with the conditions of leucocyte differentiation and production in fishes, show that the oligochaetes, as represented by *Pheretima indica*, have attained a degree of specialization which parallels that of the fishes. The lymphocytes of *Pheretima* have almost the identical morphological characteristics described for those of fishes by Jordan and Speidel ('24). Functionally, the lymphocyte has a lesser potentiality in *Pheretima*, because it cannot differentiate into an erythrocyte. It can, however, differentiate into a granulocyte. The stimulus for the production of the erythrocyte is apparently lacking in *Pheretima* and other oligochaetes. Sabin and others have claimed that erythrocyte differentiation takes place intravascularly, and at once the suggestion arises that lack of endothelial enclosure prevents the differentiation of the lymphocyte of *Pheretima* into an erythrocyte. However, if we examine the conditions of differentiation of the lymphocyte or hemocytoblast in such worms as the polychaete

Glycera, where vasa are absent, we find that the hemocyto-blasts of the hemocytopoietic organs differentiate into erythrocytes (Romieu, '23). Romieu concludes that the hemocyto-blasts are histologically similar to the free lymphocytes of the perivisceral cavity. Hence, limitation by enclosure in endothelially lined spaces is not a factor in the production of erythrocytes in annelid worms. The primary stimulus is more probably the oxygen-carbon dioxide relationship.

According to Jordan and Speidel ('24 a, p. 497), the differential stimuli which affect hemocyto-blasts in the direction of granulocytopoiesis are the "products of tissue destruction by bacteria (or bacterial toxins) and the products of tissue destruction by parasites and their toxins." In *Pheretima* it has been shown that the leucocytopoietic organs, in which the eosinophils predominate and evidently originate, are the sites of infection by the protozoan parasite *Monocystis*. Thus, a similar stimulus for the differentiation of the hemocyto-blasts into eosinophils seems to be present in *Pheretima* as in the vertebrates. Morphologically and tinctorially, the eosinophilic granulocytes of *Pheretima* are strikingly like those of the fishes and salamander as described by Jordan and Speidel.

The eosinophilic granulocytes of *Pheretima* are of two types, those with fine granules and those with coarse granules. The finely granular type is less numerous than the coarsely granular type. Both types are found in the leucocytopoietic organs. In respect to the size of the granules, the eosinophilic granulocytes are like those of the skate and certain teleosts (Jordan and Speidel, '24). In the leucocytopoietic organs I have shown that basophilic granulocytes, metachromatic granulocytes, and both types of eosinophils lie side by side in the leucocytopoietic cords. The granules of both the basophilic and metachromatic type are similar in size to those of the finely eosinophilic granulocytes, and the suggestion is made that the basophil and metachromatic granules are stages in the ripening of the finely granular eosinophils. Such a condition of differentiation of the eosino-

phils parallels that found by Jordan and Speidel in the frog ('23), by Downey ('15) in the bone marrow of the guinea-pig, and by Prenant ('22) in the parenchyma of young tri-clads. However, the relationship between the coarsely granular eosinophils and the basophil granulocytes as observed in the frog is lacking in *Pheretima*. The only relationship which can be detected is that between finely and coarsely granular eosinophils. The finely granular type is regarded as a younger stage of the coarser type. Small and large eosinophilic granulocytes are described by Kollmann ('08, p. 173) in three species of *Lumbricus*, but there is no difference recorded as to the size of the granules. In these granulocytes the number of granules increases with the size of the cell. Differentiation of the eosinophilic granulocytes of *Pheretima* by way of a basophilic stage is not the only method, since many small cells with oxyphilic granules are found both in the perivisceral cavity and in the leucocytopoietic organs. Thus, morphologically, tinctorially, and developmentally, the eosinophilic granulocytes of *Pheretima* resemble those of the lower vertebrates.

In the worm *Pheretima*, as in other oligochaetes, there is very little mesenchyme. The body wall is composed of epithelial tissue and muscular tissue bound together by flattened connective-tissue cells. These connective-tissue cells have the same morphological and tinctorial characteristics as the lining peritoneal cells of the perivisceral cavity. They have a content of deeply basophilic globules of irregular size, together with a number of metachromatic rodlets. The cells within the leucocytopoietic organs, however, have a clear cytoplasm, are more rounded, and have the general characteristics of the lymphocyte. It has already been pointed out that these cells are either free or attached, and that they either arise from the covering epithelium of the leucocytopoietic organs or they enter the leucocytopoietic organs from the perivisceral cavity. These cells, whatever their origin, are regarded as the hemocytoblasts from which the eosinophils differentiate. From this point of view, we can regard

the conditions of the origin and fate of the cells of the leucocytopoietic organs of *Pheretima* as similar to those of the hemocytopoietic organs of the vertebrates. Cells of either local origin from the mesenchyme or cells carried into these organs by the blood and lymph streams become hemocyto-blasts. They are morphologically identical, whatever their origin, and they have equal potentialities for differentiation. The direction of differentiation seems to depend upon the environmental conditions.

In *Pheretima indica*, as in the Lumbricidae examined by Kollmann ('08), all sizes of eosinophilic granulocytes are found free in the perivisceral cavity. Kollmann (p. 173) states that he has found all stages between the eosinophilic granulocytes and his leucocytes of stage I (lymphocytes of my description). Romieu ('23, p. 252) states that the granulocytes in the polychaetes are derived from "cellules ayant des caractères de lymphocytes ou érythroblastes lymphoïdes." The granulations appear very early—a condition which he says is contrary to that given by Kollmann, "qui," states Romieu, "considéraient les granulocytes chez Annélides comme les leucocytes du stade II âgés, se chargeant tardivement granulations." Such a statement is not fair to Kollmann, who distinctly states on page 173, regarding the origin of the young eosinophilic granulocytes in the oligochaetes: "Je ne vois pas les caractères de dégénérescence de ces cellules, tandis que je trouve tous les intermédiaires entre elles et les leucocytes au stade I." Such possibilities of origin of the granulocytes from the free lymphocytes are indicative of the derivation of the hemocyto-blasts of the leucocytopoietic organs from wandering lymphocytes. These organs are favorable for the maturing of a cell, since they are out of the main stream of the body fluid. The cells are securely held in the pockets of the organs and can differentiate until they are ready to emerge and become active. Such an interpretation of the genetic relationships between the lymphocytes and the granulocytes suggests that the conditions of granulocytopoiesis in the annelids are similar to those in the

Platyhelminthes and the vertebrates. The lymphocyte is interpreted as a wandering hemocytoblast, arising originally from mesenchyme in the early ontogenetic stages of the higher Metazoa and from the reticulo-endothelial system in the later stages of development. The direction of differentiation of the hemocytoblast is determined by the pressure of local needs.

During the course of this investigation, attention became directed to the methods by which the leucocytes of the perivisceral cavity and the leucocytopoietic organs reproduced. In none of the many smears and sections of the organs studied was there any evidence of mitosis. On the other hand, both in smears and sections cells with polymorphic and double nuclei were found. Cuénot ('91) held the view that these cells in the oligochaetes were dividing by amitosis, but Rosa ('96) and Kollmann ('08) stated that these were merely expressions of changes in nuclear shape and that the cells divided by mitosis. It would seem, if mitosis were the rule, that at least one cell in the many thousands observed in this study of *Pheretima* would show a mitotic figure. In the absence of such a condition and in the presence of polymorphic nuclei, not normal for the lymphocytes and hemocytoblasts, and the presence of double nuclei, I am led to conclude that the leucocytes reproduce by amitosis.

In conclusion, I venture to add to the phylogenetic history of the hemocytopoietic organs as suggested by Jordan and Speidel ('24 a) the probable course of events in the invertebrates. Starting with such a tridermic phylum as the Platyhelminthes, where the body is largely mesenchymatous and the primitive lymphocytes are wandering mesenchyme cells, there is no definite locus for hemocytopoiesis, but there is a generalized proliferation of leucocytes similar to that which obtains in the embryonal mesenchyme of vertebrates and annelids. In the next higher stage, as represented by the nemertines, definite closed vascular channels and small perivisceral spaces are present. The lymphocytes, produced in the same manner as in the non-vascular Platyhelminthes, may

enter the vasa and, as hemocytoblasts, differentiate into erythrocytes. The immediate stimulus for the production of erythrocytes with hemoglobin, appearing in this group for the first time in the animal series, is probably a greater need for oxygen than is supplied by the surrounding environment at all times. The erythrocytes may be considered as oxygen reservoirs.

The conditions of organization in the polychaetous annelids represent the next higher stage in the phylogeny of the hemocytopoietic organs. The vasa become more complex, respiratory pigment is more abundant, the perivisceral spaces become larger, but the lining cells, which may now be termed peritoneal cells, retain their ancestral hemocytoblastic potentialities and bud off cells into the perivisceral cavity. According to Romieu, some of these cells may enter the vasa and produce the respiratory pigment, which is withdrawn from them by the hemolytic activity of the fluid within the vasa. There are no definite highly developed hemocytopoietic organs.

The next higher stage of hemocytopoietic localization may be represented by the conditions in *Pheretima indica*. Here are present leucocytopoietic organs. These are definitely organized outgrowths of the septa. They contain differentiating hemocytoblasts and specialized areas of hemocytoblastic peritoneal epithelium. These organs are close to the dorsal mesentery and nephridia. They either produce hemocytoblasts or they afford a locus for the differentiation of lymphocytes (hemocytoblasts) which are carried into their lumina. Granulocytes particularly are produced here.

In the change from this type of organization of the leucocytopoietic organs to that found in the lower fishes, it is conceivable that the segmental septa disappear, and the leucocytopoietic organs in part fuse and become enclosed in the dorsal mesentery, forming a primitive spleen, or in part become involved with the developing mesonephros and form the intertubular hemocytopoietic tissue. The amount of mesenchymal connective tissue increases greatly. Lymphatic

spaces and vessels appear. The blood-vascular system becomes more extensive and more complex, correlated with its increasingly important functions in vertebrates. Open sinuses continuous with the reticular stroma appear in the neighborhood of the primitive blood-forming organs (i.e., the spleen and intertubular region of the kidney). Thus, easy direct access into the blood vessels is afforded the cells produced by the hemocytopoietic organs.

In worms only a few blood cells (leucocytes) get into the blood vessels, and these by process of diapedesis. The rest perform their function in the perivisceral cavity. Since the hemocytopoietic organs are present in each segment (except a few anterior ones) and since the septa are perforate, it is obvious that the blood vessels are not needed for the distribution of the leucocytes. The cells themselves can live in either environment, blood vessels or perivisceral cavity, but, since they are produced by the peritoneal epithelium and their functions are largely concerned with the needs of the serous spaces and the bodily tissues in general, the number entering the vasa is small.

In vertebrates, however, the conditions are reversed, and the cell products of the hemocytopoietic organs go into the blood vessels almost entirely. Both erythrocytes and leucocytes are cell products of the vertebrate (fish) hemocytopoietic organs. The red cells perform their function entirely within the closed vessel system. The white cells form a permanent constituent of the circulation, some of them migrating out from the vessels to perform their function among the tissues or in the cavities of any region of the body. Since in vertebrates the leucocyte-producing organ is not as extensive as in *Pheretima*, but is sharply localized, it is obvious that the blood vessels perform a valuable function in aiding the distribution of the leucocytes to distant regions of the body.

This comparison of *Pheretima* and the fish suggests, therefore, that in the evolution of the blood-forming organs a shift has occurred from a series of leucocytopoietic organs that

pour their product into the perivisceral cavity to a leuco-erythrocytopoietic organ that pours its product into the blood vessels.

SUMMARY

1. The perivisceral fluid of *Pheretima indica* contains five types of leucocytes: the lymphocytes, the monocytes, the granulocytes, the lamprocytes, and the linocytes.

2. The granulocytes differentiate either from free lymphocytes, from peritoneal epithelial cells lining the leucocytopoietic organs, or from lymphocytes (hemocytoblasts) embedded in these organs. The lymphocyte is a hemocytoblast.

3. The eosinophilic granulocyte is the most numerous of the several types of granulocytes. Morphologically and tinctorially, it resembles the eosinophil of the fishes. The granule of the eosinophilic granulocyte may pass through a stage of ripening in which it is at first small and basophilic. This stage is followed by a metachromatic stage, which culminates in the eosinophilic stage. Eosinophilic granules may appear directly in the hemocytoblast without passing through a ripening process. Young eosinophilic granules are small and homogeneous, while older granules are large and the tinctorial substance tends to accumulate on one side of the granule.

4. The stimulus for the excessive production of eosinophils is thought to be the degree of infection of the leucocytopoietic organs by the gregarine *Monocystis* sp.

5. The monocytes are developed in the perivisceral cavity by growth of the lymphocytes.

6. The lymphocytes and monocytes are phagocytes.

7. The conditions of leucocyte differentiation parallel those of fishes.

8. The leucocytopoietic organs are a series of bilaterally symmetrical structures which have arisen by complex foldings of the cephalic faces of the septa. They lie on either side of the dorsal blood vessel and attached to it. The members of each pair are connected with each other above the dorsal vessel. The lumina of these organs are continuous

with the lumen of the perivisceral cavity. Each lumen consists of a large central cavity, from which ramifies a series of radiating canals which end blindly in masses of hemocytoblasts, the leucocytopoietic cords. The stroma of the organ is composed of muscle fibers continuous with the muscle fibers of the septum. The surface of the organ is covered with flattened, undifferentiated, peritoneal cells. The leucocytes are differentiated from the hemocytoblasts of the leucocytopoietic cords.

9. There is no evidence of mitosis in any of the cells of the perivisceral cavity nor of the leucocytopoietic cords. But lymphocytes, monocytes, eosinophilic granulocytes, and hemocytoblasts of the leucocytopoietic cords, with two nuclei, have been observed. Polymorphic nuclei are also present in the hemocytoblasts of the leucocytopoietic cords. Hence, it is concluded that the leucocytes and hemocytoblasts of *Pheretima indica* increase by amitosis.

10. Lamprocytes, special leucocytes of large size, containing globules with a complex tinctorial reaction, are found in *P. indica* for the first time outside of *Octochaetus multiporus*. Their function is unknown, but it is suggested that they are excretory in character.

11. The linocytes, another problematical group of leucocytes, containing large vacuoles or thread-like masses, are also described for the first time since their discovery in *O. multiporus*. Their function is unknown.

12. The genetic relationships between the leucocytes and the peritoneal epithelium in *P. indica* are comparable to the genetic relationships between the mesenchyme and the leucocytes in the fishes, but the conditions in *P. indica* can only be regarded as paralleling the conditions of leucocyte differentiation in the lower vertebrates and not as preceding them phylogenetically.

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PLATES

DESCRIPTION OF FIGURES

All of the figures illustrating this paper were drawn with the aid of a camera lucida, except figure 40, which was drawn from a dissection under the binocular microscope.

PLATE 1¹

EXPLANATION OF FIGURES

- 1 Lymphocyte from smear of perivisceral fluid. Giemsa. $\times 1300$.
- 2 Lymphocyte from smear of perivisceral fluid. Note pseudopodia. Giemsa. $\times 1300$.
- 3 Lymphocyte with two nuclei. Smear. Giemsa. $\times 1300$.
- 4 Monocyte. Smear. Giemsa. $\times 1600$.
- 5 Lymphocyte as seen in perivisceral cavity. Helly fixation, eosin-azur II stain. $\times 1300$.
- 6 Monocyte as seen in perivisceral cavity. Helly, eosin-azur II. $\times 1300$.
- 7 Monocyte. Smear. Note pseudopodia of petaloid type. Giemsa. $\times 1600$.
- 8 Monocyte with two nuclei. Smear. Giemsa. $\times 1600$.
- 9 Small eosinophilic granulocyte. Smear. Giemsa. $\times 1300$.
- 10 Small eosinophilic granulocyte as seen in perivisceral cavity. This type of granulocyte has large granules in which the tinctorial material is condensed on one side of the granule. Helly, eosin-azur II. $\times 1300$.
- 11 Small eosinophilic granulocyte. Smear. Giemsa. $\times 1300$.
- 12 Eosinophilic granulocyte of same type as shown in figure 10, but of larger size and with more granules. From the perivisceral cavity. Helly, eosin-azur II. $\times 1300$.
- 13 Large eosinophilic granulocyte. Smear. Giemsa. $\times 1300$.
- 14 Large eosinophilic granulocyte with two nuclei. Smear. Giemsa. $\times 1300$.
- 15 Eosinophilic granulocyte with small granules as seen in perivisceral cavity. Helly, eosin-azur II. $\times 1300$.
- 16 Lamprocyte. Smear. Wright's blood stain. $\times 1500$.
- 17 Lamprocyte as seen in perivisceral cavity. Note differential tinctorial zones in globules. Helly, eosin-azur II. $\times 1300$.
- 18 Peritoneal epithelial cell. Smear. Wright. $\times 1500$.
- 19 Linocyte. Smear. Wright. $\times 1500$.
- 20 Metachromatic granulocyte. Smear. Giemsa. $\times 1300$.
- 21 Basophilic granulocyte as seen in perivisceral cavity. Helly, eosin-azur II. $\times 1300$.
- 22 Granulocyte with basophilic and metachromatic granules from leucocytopoietic cord. Helly, eosin-azur II. $\times 1600$.
- 23 Granulocyte with dark metachromatic granules from leucocytopoietic cord. Helly, eosin-azur II. $\times 1600$.
- 24 Granulocyte with light metachromatic granules from leucocytopoietic cord. Helly, eosin-azur II. $\times 1600$.
- 25 Segment of a leucocytopoietic cord. *Bo.*, basophilic granulocyte or young eosinophil; *Eq.m.*, granulocyte containing both metachromatic and basophilic granules, an intermediate stage in the ripening of the eosinophilic granules; *Eq.r.*, granulocyte with granules more acidophilic, but still metachromatic, a late stage in the ripening process; *Eq.r'*, granulocyte with ripe eosinophilic granules; *L.*, hemocytoblast; *Pe.*, peritoneal epithelial cell. Helly, eosin-azur II. $\times 1600$.

¹The extra cost of this color plate was met by a grant from the Research Committee of the University of Virginia.

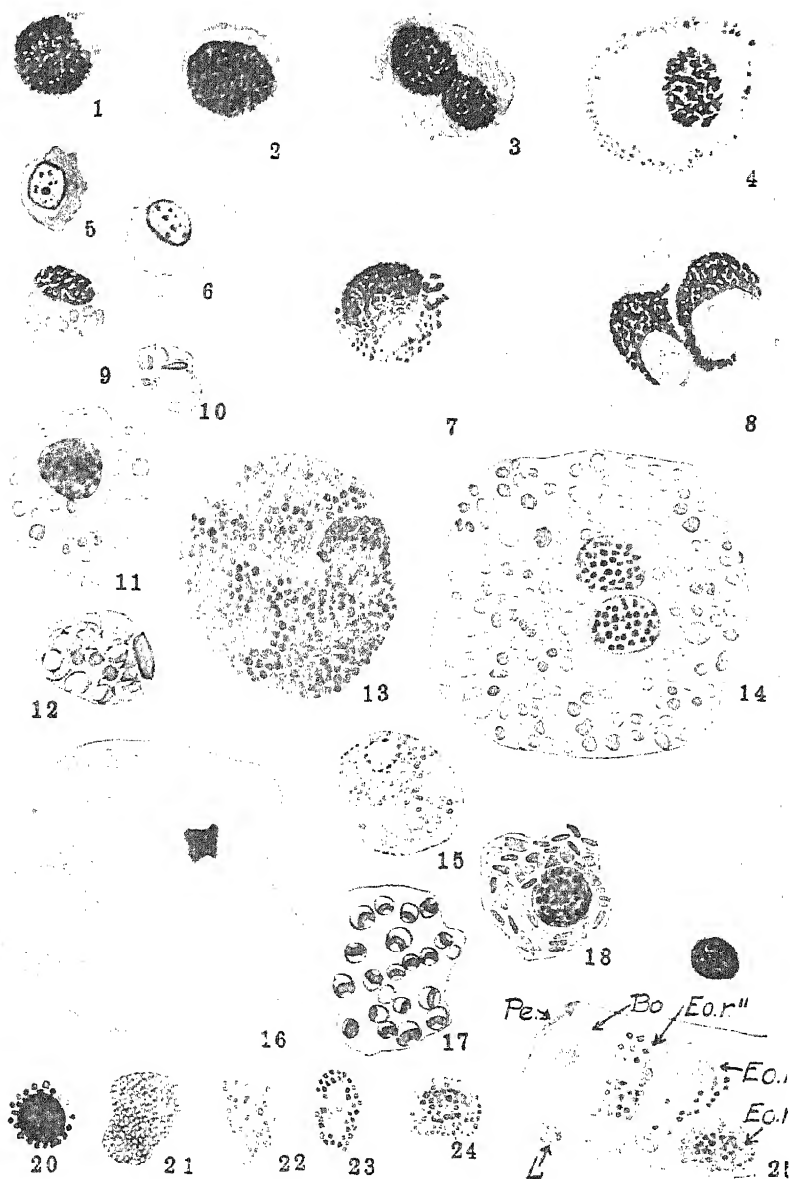


PLATE 2

EXPLANATION OF FIGURES

- 26 Lymphocyte in vitro. Note short filiform pseudopodia. $\times 1300$.
- 27 Lymphocyte in vitro. $\times 1300$.
- 28 Lymphocyte in vitro, showing elongate shape. $\times 1300$.
- 29 Lymphocyte in vitro from perivisceral fluid of worm injected with Chinese ink in Ringer's solution for forty-five minutes. The small black particles are ingested ink particles. $\times 1300$.
- 30, 31, and 32 Three views of the same monocyte in vitro, drawn at intervals of fifteen seconds to show changes in the shape of the petaloid pseudopodia. $\times 1300$.
- 33 Monocyte in vitro from perivisceral cavity of worm injected with Chinese ink in Ringer's solution for forty-five minutes. $\times 1300$.
- 34 and 35 Linoocytes in vitro. Note the lobe-like projections caused by the disintegrating thread-like content. $\times 1500$.
- 36 Granulocyte in vitro. Note the small, transparent granules. $\times 1300$.
- 37 Linoocyte as seen in perivisceral cavity. The nucleus is slightly basophilic, the cytoplasm faintly basophilic, and the vacuoles unstained. Helly, eosin-azur II. $\times 1300$.
- 38 and 39 Two stages in the degeneration of the lamprocyte. Figure 38 is an earlier stage than figure 39. The globules are shrunken and only faintly stained, the cytoplasm is vacuolated, and the surface of the cell is wrinkled. Helly, eosin-azur II. $\times 1300$.
- 40 Dorsal view of dissection of several segments from the posterior fifth of the body of *Pheretima indica*, showing the positional relationships of the bilaterally symmetrical leucocytopoietic organs. The anterior end is toward the top of the page. *D.V.*, dorsal blood vessel; *L.O.*, leucocytopoietic organs; *Sp.*, septum; *Int.*, intestine. $\times 6$.

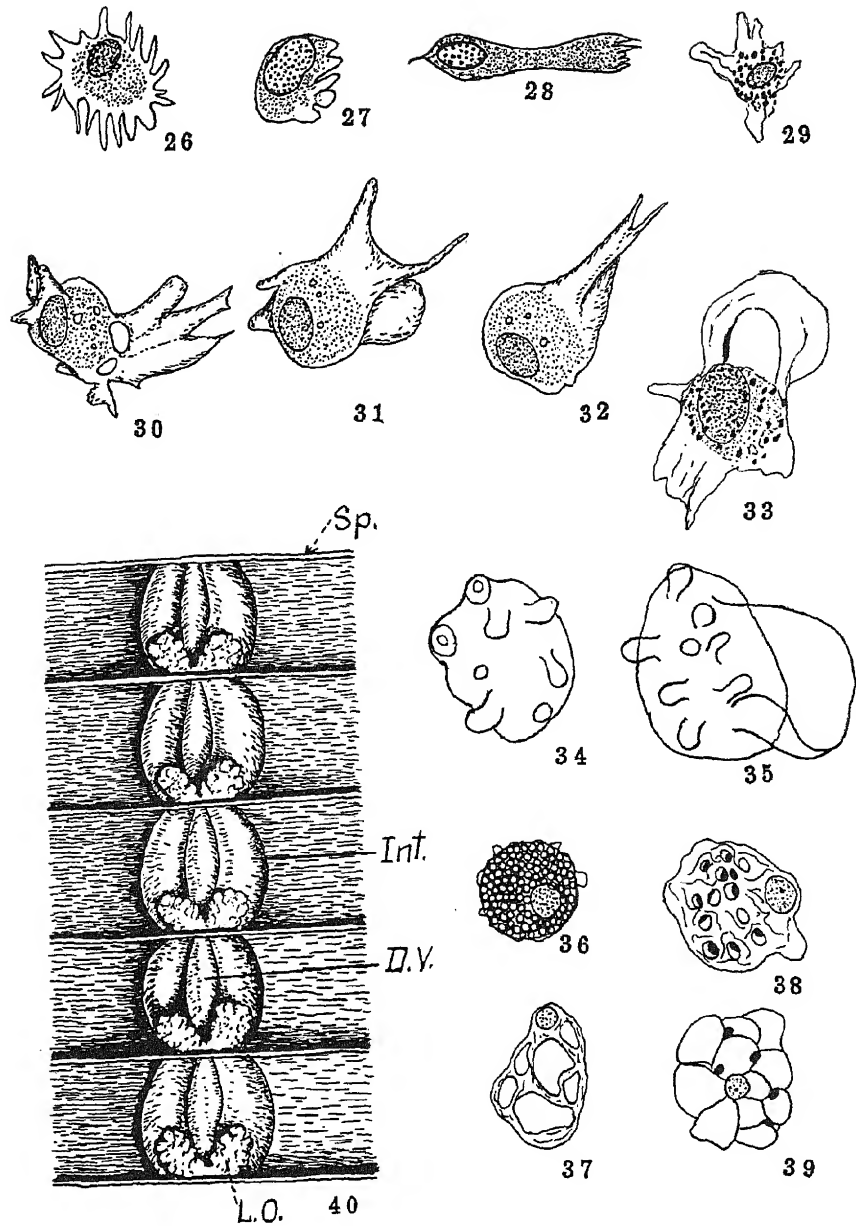


PLATE 3

EXPLANATION OF FIGURES

41 Lamprocyte in vitro. The globules in gray are light yellow in the living condition. $\times 1500$.

42 Chloragocyte from smear of teased intestinal wall. The globules are translucent, with a bluish-green tinctorial reaction. The nucleus is large, vesicular, pinkish in color, with a small bluish nucleolus. Wright. $\times 1500$.

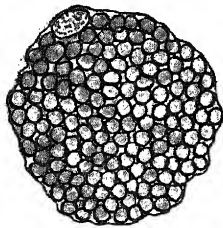
43 Young linocyte in vitro. Note the large colorless vacuole, the thin shell of cytoplasm, and the eccentrically placed nucleus. $\times 1300$.

44 Old linocyte in vitro. The cytoplasm is filled with a coiled thread-like mass. $\times 1500$.

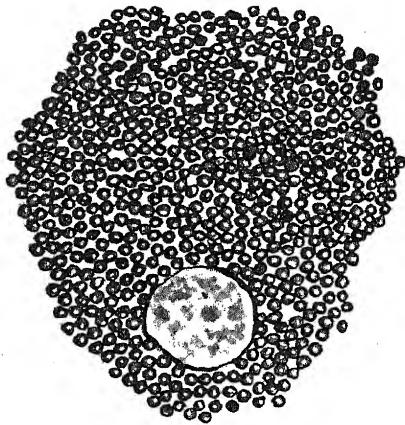
45 Linocyte from smear fixed with osmic acid. $\times 1500$.

46 Large linocyte spread out in a supravital preparation with neutral red. The spaces between the vacuoles are slightly pink; the vacuoles are not stained. $\times 1300$.

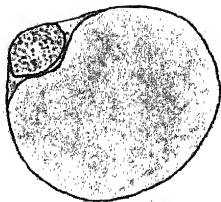
47 Section of leucocytopoietic cord, showing the polymorphic and double nuclei of the hemoctoblasts. These nuclear modifications are regarded as evidences of amitosis. Helly, eosin-azur II. $\times 1600$.



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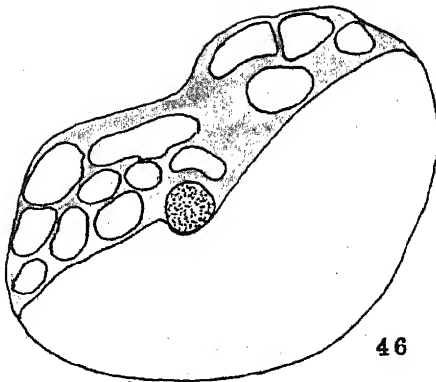
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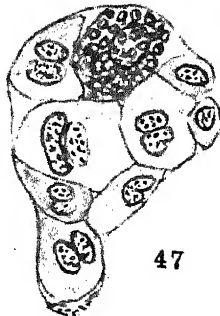
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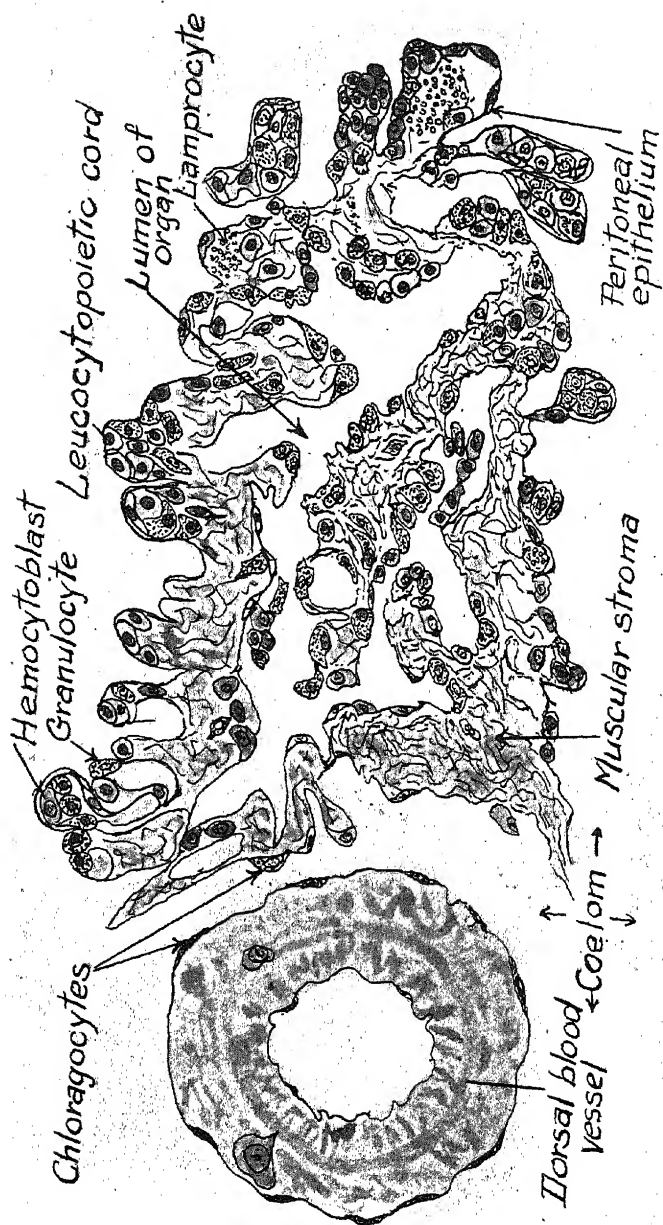
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49 Transverse section through a leucocytopoietic organ. The different types of cells are shaded as in figure 48. $\times 600$.

A STUDY OF THE FUNCTION OF THE EPIDIDYMIS

I. IS THE ATTAINMENT OF FULL SPERMATOZOON MATURITY ATTRIBUTABLE TO SOME SPECIFIC ACTION OF THE EPIDIDYMAL SECRETION?¹

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Hull Zoölogical Laboratory, The University of Chicago

FOUR CHARTS

AUTHOR'S ABSTRACT

During the past few years many functions have been assigned to the epididymis which have been supplementary to its rôle in sperm storage and to its secretory activity. One of these, the suggestion that sperm attain full maturity and are strengthened in consequence of some action of its secretion, has been reinvestigated as the first part of a study which is intended to include other aspects of the problem.

No evidence in support of the theory was obtained from a series of experiments on various mammals representing a repetition and extension of earlier work. On the contrary, the strengthening and attainment of full spermatozoon maturity would seem to be the outcome of changes which are inherent in the sperm themselves. It is suggested, therefore, until other aspects of the subject can be reinvestigated, that the epididymis is simply a reservoir for sperm in which the processes of sperm development which start while they are still contained in the testis are free to continue because of the favorable environment present in the epididymis.

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INTRODUCTION

The problem of the function of the epididymis arose as the outcome of an investigation of the histological changes which occur in the guinea-pig testis following the application of tem-

¹This work was aided by a grant from the Committee on Sex Research of the National Research Council; grant administered by F. R. Lillie.

peratures slightly higher than that of the body (Young, '27). Since becoming interested in the problem, however, the opinions with respect to epididymal function have been found to be so numerous and the methods of investigation so varied, that it has seemed advisable to investigate its different aspects separately. The experiments reported in this paper represent a study of that phase of the problem which is related most closely to my earlier work cited above. The results from the investigation of other aspects of the problem will be reported as soon as they have been obtained.

In the earlier study (Young, '27) water heated a few degrees above body temperature was allowed to run over the guinea-pig scrotum. At frequent intervals from one hour until one year after the treatment, testes were removed and studied histologically. The degenerative changes which were known to follow such a treatment (Moore, '24) were found to occur in a definite order. The actively dividing primary and secondary spermatocytes and the younger spermatids were the first cells to show degenerative changes. The older spermatids were the next to be visibly affected and, subsequently, the degeneration of spermatozoa was observed, those in the epididymis, however, being more resistant than those in the testis. Spermatogonia were among the most resistant germinal elements and many usually survived the heat injury to serve as the stem cells for a new germinal epithelium. It was shown, therefore, that male germ cells exhibit a rising and falling gradient of susceptibility to high temperature as they develop.

These observations might not have been commented upon further had it not been for a suggestion as to epididymal function advanced by Stigler ('18). He, too, had observed in the guinea-pig, rat, and mouse that sperm contained in the epididymis are more heat resistant than those in the testis. He stated, in addition, that those contained in the posterior end, of the epididymis are more heat resistant than those contained in the anterior end, and that sperm contained in the second and subsequent ejaculates of a series are less

heat resistant than those contained in the first ejaculate. He concluded that sperm experience a strengthening which increases their heat resistance and, presumably, resistance against other physiological factors as well.

His theory was referred to without comment (Young, '27, p. 492), except for the suggestion that confirmatory experiments seemed desirable. Having pointed out that the heat resistance of male germ cells increases progressively following the completion of the reduction divisions until they have attained their final form as spermatozoa, it seemed strange that some external factor such as epididymal action should have to be invoked to explain further increase in resistance during their residence in this organ. In fact, it seemed more probable that this progressive increase in resistance to high temperature is simply an expression of changes which are inherent in the germ cells.

An examination of the literature revealed no similar suggestion, except for a short statement made by von Möllendorff during the meeting of the Anatomischen Gesellschaft in Halle in 1924 and referred to by Redenz ('24, S. 607). According to von Möllendorff, the changed properties of sperm which appear after they have passed through the epididymis may depend on a change in the colloidal state of the sperm and on a transformation in their protoplasm, and not on the microscopically invisible covering which Redenz has postulated to exist as a product of the epididymal secretion. Much evidence, on the other hand, seemed to oppose this idea and that expressed by myself, and to support Stigler's theory that the strengthening of sperm during their residence in the epididymis is attributable to some action of the epididymal secretion.

Most important, perhaps, were the observations that sperm removed from the posterior levels of the epididymis and placed in physiological saline solution show a stronger movement than those removed from the anterior levels and testis (Hammar, '97; Walker, '99; Tournade and Regaud, '11; Tournade, '13, and Mettenleiter, '25). Of this group, Tour-

nade actually attributes the stronger motion of sperm from the posterior levels of the epididymis to some action of the secretion of this organ—a conclusion with which von Lanz ('24 a and b) agrees. Other observations which have been quoted in support of this theory of epididymis function were those to the effect that the second and subsequent ejaculates in a series contain fewer and less active sperm than the first (Mantegazza, '66, for man; Lewis, '11, for horses; Lloyd-Jones and Hays, '18, for rabbits; and Amantea and Krzyszkowsky, '21, and Krzyszkowsky and Pawlow, '27, for dogs).

The problem as outlined has been studied during the past months, and the results and conclusions are reported below. I am indebted to Prof. Frank R. Lillie for his assistance in making the study possible and to Prof. Carl R. Moore for his counsel and help during the progress of the work.

EXPERIMENTAL

a. The motility of sperm from the seminiferous tubules

Probably the most important evidence for the conclusion that spermatozoa attain their full maturity in consequence of some action of the epididymal secretion was the observation noted above, that sperm removed from the testis and anterior end of the epididymis are non-motile when placed in physiological saline solution, while those removed from the posterior levels are motile (Hammar, '97, for the dog; Tournade and Regaud, '11, for the rat; and Tournade, '13, for the rat, rabbit, guinea-pig, and dog). It seemed desirable to reinvestigate this point.

Sperm from the seminiferous tubules of three bulls, five rams, four guinea-pigs, and four rats were examined following the maceration of small fragments of the testis in Locke's solution. In the bull, guinea-pig, and ram some sperm from each testis were observed to exhibit a weak but nevertheless distinct vibratile movement of the tail. In the rat many sperm were found to be motile, a strong progressive motion being shown. These observations do not agree with those of Hammar, Tournade, and Regaud, but they are con-

sistent with those reported by Walker ('99), who found that sperm from the testis of the dog can be activated slightly by the addition of prostatic secretion.

Sperm from the head, body, and tail of the epididymis of the bull, ram, and rat were examined next. The impression is gained that motion is strongest in the tail and weakest in the head—observations which are in agreement with those of Hammar, Tournade and Regaud, and Walker. The point should be confirmed, however, by more precise methods than have been used thus far.

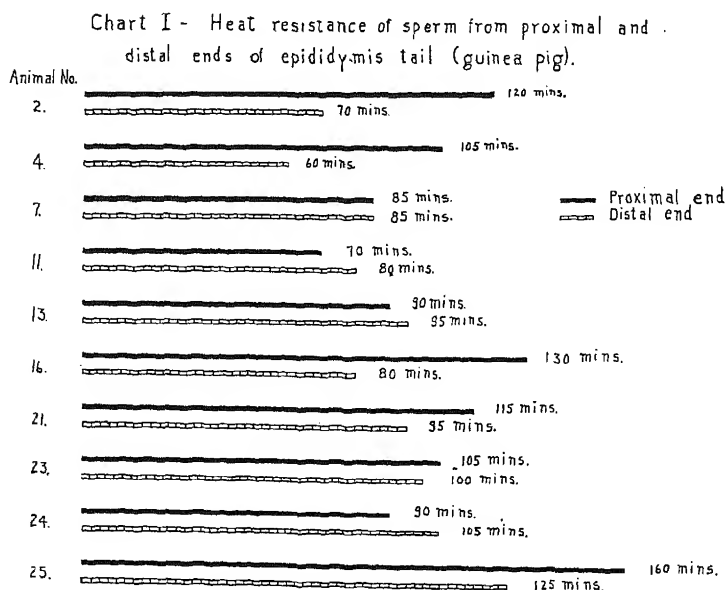
It is seen, then, that although the capacity for motion appears to become strengthened as sperm pass through the epididymis, that its first appearance can be observed while the sperm are still contained in the testis. The observation is interpreted as indicating that the acquisition and strengthening of motility is not attributable to any specific action of the epididymal secretion, but is an expression of sperm development, the capacity for which is inherent in the spermatozoon itself.

b. The resistance of sperm from different levels of the epididymis to high temperature

In the second group of experiments directed toward an investigation of the theory that spermatozoa attain their full maturity in consequence of some action of the epididymal secretion, a study of the heat resistance of sperm from different parts of the epididymis was made. It will be recalled that Stigler heated sperm removed from the testis and epididymis as well as sperm obtained from successive ejaculates and found, in the first case, that those from the testis are less heat resistant than those from the epididymis, and, in the second case, that sperm from the second and subsequent ejaculates are less resistant than those from the first. To the writer it seemed justifiable and fully as accurate to remove sperm from the proximal and distal ends of the epididymis, place them in Locke's solution in as nearly equal concentration as possible, subject them to a given simultaneous tem-

perature exposure in a water-bath, and observe the moment when motion is no longer visible on the part of any one sperm. A temperature of 46°C. was chosen after some preliminary trials.

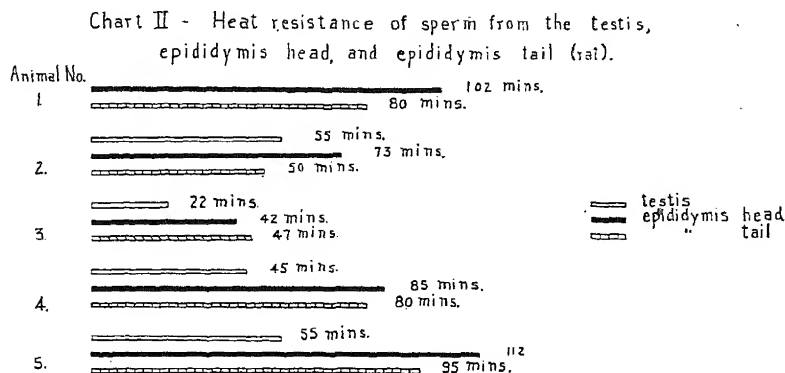
Spermatozoa were removed from twenty-five guinea-pigs and studied in this manner. The data from ten typical experiments are shown in chart 1.



The result was that, contrary to what was expected on the basis of Stigler's work, sperm from the proximal end of the epididymis were found to be more resistant when exposed to high temperature than sperm from the distal end. The average limit of motion of sperm from the proximal end, compiled from the records of twenty-five guinea-pigs, was 105 minutes, while the average limit of motion of sperm from the distal ends of the epididymides was only 76 minutes. Sperm from the proximal end were conspicuously more heat resistant in fifteen cases, there was but little difference in six cases, and

sperm from the distal end were slightly more heat resistant than those from the proximal end in four cases.

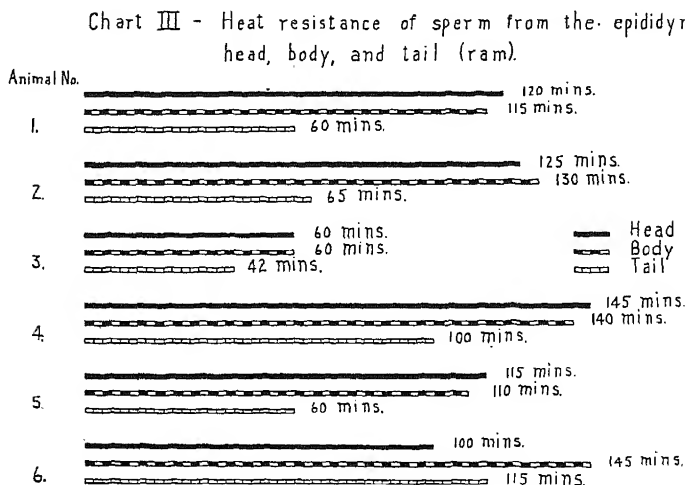
The unexpected result suggested a repetition of the experiment using sperm from other species, and the rat and ram were selected. Both provide better material for such an experiment than the guinea-pig. The head, body, and tail of the epididymis are sharply divided in both species, and, in the rat, sperm from the testis are so motile when placed in Locke's solution that they, too, can be compared with sperm from the two parts of the epididymis. The data for sperm from the testis, epididymis head, and epididymis tail of five rats are shown in chart 2.



The agreement with the results obtained when guinea-pig sperm were used is perfect. Sperm from the epididymis head were motile for as long as eighty-three minutes, averaged for the five animals, while those from the epididymis tail were motile for only seventy minutes. Sperm from the epididymis tail were more heat resistant than those from the epididymis head in only one out of five cases. Sperm from the testis were the least heat resistant, the average limit of motion being only forty-four minutes.

The data obtained from eight rams when sperm from the epididymis head, body, and tail were heated separately are shown in chart 3.

Results which are consistent with those obtained previously by myself on the guinea-pig and rat and contradictory to those reported by Stigler were obtained. The average limit of motion on the part of sperm from the epididymis head was 107 minutes, on the part of sperm from the epididymis body it was 122 minutes, and on the part of sperm from the epididymis tail it was only 78 minutes. In the ram, sperm from the tail, located distally, were found to be less resistant than those from the proximally located body in every animal,



and less resistant than those from the epididymis head in seven out of eight animals.

This apparently satisfactory demonstration that sperm from the anterior portion of the epididymis of the guinea-pig, rat, and ram are more resistant when exposed to high temperature than sperm from the posterior portions of the same epididymides raised the following question: How are data from these experiments in which sperm from two ends of the epididymides were heated directly to be reconciled with the results obtained by Stigler, who heated sperm from successive ejaculates?

Final answer to the question must await the opportunity to repeat in every detail the procedure followed by Stigler. In the meantime, however, it is thought that observations which have been reported by other investigators may suggest the answer. The work of Mantegazza ('66), Lewis ('11), Lloyd-Jones and Hays ('18), Amantea and Krzyszkowsky ('21), and Krzyszkowsky and Pawlow ('27), in which attention is called to the reduction in the number of sperm contained in successive ejaculates, has been cited. It seems likely from this that the number of sperm in the second ejaculate may have been less than the number in the first ejaculate in the experiment performed by Stigler. Simultaneously, the idea was suggested that the greater dilution of sperm in the second ejaculate may be a factor of some importance. Barthélémy ('26) has shown that the duration of motion of frog sperm is longer when the concentration of sperm is increased, and Gray ('28) has shown that *Echinus* sperm live longer in concentrated suspensions than they do in dilute suspensions.

A simple experiment was arranged to test the point for the guinea-pig. Epididymides were macerated in about 25 cc. of Locke's solution and the sperm suspension poured off. This suspension was considered to be full strength and various dilutions were made by adding one or more parts of Locke's solution to a constant volume of sperm suspension. In this way dilutions of $1/2$, $1/3$, $1/4$, $1/7$, $1/8$, $1/14$, and $1/25$ were obtained. Test-tube samples were then heated in a water-bath at 46°C ., and the time recorded when the last motile sperm was observed. The results are shown in table 1.

The decrease in resistance to high temperature with increased dilution is clearly demonstrated for guinea-pig sperm. Of course, it cannot be known that a greater dilution of sperm in the second ejaculate than in the first will likewise explain the decreased heat resistance of sperm contained in the second and subsequent ejaculates until Stigler's experiments can be repeated. In the meantime, however, it is difficult to account for the lower resistance of the sperm contained in the second ejaculate in any other way, when it has been demonstrated on

so many animals that sperm in the distal end of the epididymis are generally less heat resistant than those from the proximal end.

The significance of this group of experiments for the problem of the function of the epididymis may be summarized as follows:

1. Spermatozoa removed from the distal portion of the epididymis have been found to be less heat resistant than those removed from the proximal portion. The theory, therefore, that spermatozoa become progressively more heat re-

TABLE 1

Effect of dilution on duration of motion of guinea-pig sperm maintained at 46°C.

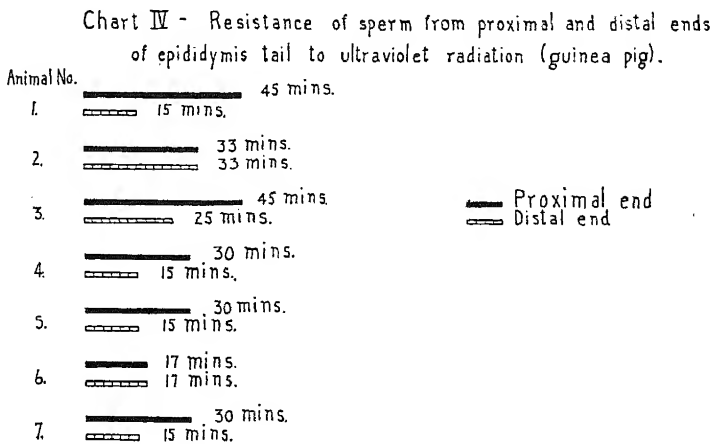
EXPERIMENT NO.	PARTS OF LOCKE'S SOLUTION TO UNDILUTED SPERM SUSPENSION							
	Undiluted	1:1	2:1	3:1	6:1	7:1	13:1	24:1
	<i>minutes</i>	<i>minutes</i>	<i>minutes</i>	<i>minutes</i>	<i>minutes</i>	<i>minutes</i>	<i>minutes</i>	<i>minutes</i>
1	105	105		105		70		
2	100	120		110		60		
3	105		85		55			25
4	90		60		25		25	
5	80		70		50		30	
6	70		40		30		20	

sistant as they pass through the entire length of the epididymis should be abandoned.

2. There is some increase in heat resistance as sperm pass through the proximal portion of the epididymis lumen; at least this is true in the ram, where sperm from the body have been shown to be more heat resistant than those from the head. It has been shown, however (Young, '27), that this change in heat resistance begins while the germ cells are still contained in the testis and, in addition, in this study, that the increase in heat resistance is not continued as sperm pass through the body into the tail. On the contrary, sperm contained in the tail are less heat resistant than those in either the body or head. For these reasons, therefore, changes in heat resistance which do occur in sperm during their resi-

dence in the epididymis probably should be conceived as being independent of any specific action by its secretion.

3. It is suggested that the lower resistance of sperm from the second and subsequent ejaculates of a series to high temperature, as reported by Stigler, may be attributable to a greater dilution of sperm in these ejaculates than in the first, rather than to the immaturity of the sperm.



c. The resistance of sperm from different levels of the epididymis to ultraviolet radiation

A third series of experiments, arranged for the purpose of reinvestigating the theory that the strengthening of sperm as they pass through the epididymis is attributable to some specific action by the epididymal secretion, was similar to the second series, except that sperm from different levels of the guinea-pig epididymis were exposed to ultraviolet radiation instead of to high temperature. Unscreened radiation from a quartz mercury-vapor arc running at 110 volts, D.C., at $4\frac{1}{2}$ amperes was employed. Exposures were made at a distance of 44 cm. from the center of the arc. The results are shown in chart 4.

Except for the much shorter duration of motion on the part of all sperm, the results are no different from those obtained when sperm were exposed to high temperature. In six out of eight cases sperm from the proximal end were more resistant than those from the distal end, in two cases no difference was observed, and sperm from the distal end were not more resistant than those from the proximal end in a single experiment. The average limit of motion by sperm from the proximal end of the epididymis was thirty-three minutes, while those from the distal end remained motile for an average of only nineteen minutes.

Stigler's suggestion, therefore, that sperm resistance against other physiological factors as well as high temperature increases as they pass through the epididymis has not been confirmed by the employment of ultraviolet rays.

DISCUSSION

The experiments which have been described represent an effort to solve one of the problems involved in a study of the function of the epididymis. Several workers, but particularly Stigler, and Tournade and Regaud, have accepted observations of a diverse nature as evidence for the conclusion that the secretion of the epididymis exerts a strengthening action on spermatozoa which is manifested by a stronger capacity for motion and by an increased resistance against high temperature and other physiological factors as well. Many of the experiments from which this suggestion followed have been repeated and supplemented, but no evidence has been obtained which can be regarded as confirming the conclusions of these earlier investigators.

For example, Hammar, and Tournade and Regaud state that sperm are non-motile in the testis and anterior levels of the epididymis, but strongly motile in the posterior levels—from which it has been concluded that sperm acquire their capacity for motion as they pass through the epididymis and as a result of some action of its secretion. My own observations on four species and sixteen individuals indicate

that sperm's capacity for motion becomes strengthened as they pass through the epididymis, but also that many sperm acquire their capacity for motion while they are still contained in the testis. From this it is concluded that at least one manifestation of sperm strength, namely, the capacity for motion, is acquired independently of any specific action of the epididymal secretion.

Stigler has cited experimental evidence in support of the statement that spermatozoa contained in the distal end of the epididymis remain motile longer when exposed to high temperature than those from the proximal end of this organ. From this he concluded that the epididymal secretion exerts a strengthening influence on sperm which increases their resistance against high temperature and presumably against other physiological factors as well. The observations of Hammar, and Tournade and Regaud, described above, have been quoted in support of his suggestion and, in addition, the observations of Mantegazza, Lewis, Lloyd-Jones and Hays, Amantea and Krzyszkowsky, and Krzyszkowsky and Pawlow, who have reported that sperm in the second and subsequent ejaculates of a series remain motile for a shorter time than those contained in the first ejaculate.

When the experiments arranged for a reinvestigation of this work had been completed, it was found that sperm from the distal end of the epididymides from forty-nine animals of three species were actually less heat resistant than those from the proximal ends. Likewise, when sperm were removed from the epididymides of eight guinea-pigs and exposed to continuous ultraviolet radiation, the same situation prevailed; sperm from the distal end became non-motile sooner than those from the proximal end.

The results certainly do not provide a demonstration that the secretion of the epididymis exerts a strengthening influence on sperm as suggested by Stigler. They do suggest, however, either that sperm are not constantly strengthened as they pass through the epididymis or that resistance to high temperature and ultraviolet radiation are not necessarily

expressions of sperm strength. The first possibility is not consistent with the observation that the capacity for motion, considered generally to be an expression of sperm strength, seems to become stronger as sperm pass through the epididymis. Furthermore, it would lead to the awkward conclusion that sperm in the middle of the epididymis are stronger than those about to be discharged. The second possibility, on the other hand, that resistance to high temperature and ultraviolet radiation are not necessarily expressions of sperm strength seems more probable and is the conclusion I have reached. There is nothing which discredits this suggestion in any of the experiments which have been reported. In addition, a strengthening in the sense of an increased capacity for motion may well be more important in the attainment of the egg than a strengthening in the sense of an increased resistance against high temperature and ultraviolet radiation.

The idea that it is capacity for motion which is a measure of sperm strength rather than resistance against high temperature suggests an explanation for the lower resistance of sperm removed from the distal end of the epididymis to both high temperature and ultraviolet radiation. It may be that the more active sperm found in the distal end are more susceptible to the destructive action of these two agents than the less active cells from the proximal end.

Less attention has been given to the observations of Mantegazza, Lewis, and the others on the lower number and vitality of sperm contained in the second and subsequent ejaculates of a series—from which it likewise has been concluded that the epididymis exerts a strengthening influence on sperm—than on the observations of Stigler. The importance of sperm density in any study of sperm vitality has been pointed out, however, and it would seem well, on this account, to withhold any conclusion as to epididymal function which is based on the vitality of sperm from successive ejaculates until the factor of sperm density has received further attention.

In the discussion of the experiments which have led to the conclusion that the epididymal secretion exerts no specific action on the strengthening of sperm as they pass through it, there has been no minimization of the importance of the epididymal secretion as a medium in which sperm are preserved during the maturing or ripening process which continues after they have been carried out of the testis. Attention has simply been directed to recently accumulated evidence against the theory that the stimulus which induces these maturing or ripening changes in sperm originates in the epididymis. When the capacity for motion is regarded as an expression of sperm strength, any action of the epididymal secretion in strengthening the capacity for motion is felt to be excluded by the fact that the capacity for motion may be gained before the sperm leave the testis. For those who have considered heat resistance of sperm to be a manifestation of sperm strength, any action of the epididymal secretion in strengthening sperm is felt to be excluded, 1) by the fact that the heat resistance of germ cells begins to increase before they have left the testis, and, 2) by the fact that spermatozoa in the distal end of the epididymis are generally less heat resistant than those in the proximal portion.

A last point for discussion concerns the present status of the problem. As was stated in the beginning, the experiments reported in this paper were directed toward the solution of only one of the questions involved in a study of the function of the epididymis. Until the other aspects of the problem can be investigated, only a tentative hypothesis of the relationship between the epididymis and the spermatozoa contained in it can be given. It is reemphasized that the epididymis is essentially a reservoir for sperm. In it the processes of sperm development which started while they were still attached to the germinal epithelium are free to continue by reason of the suitable environment present in the organ. It has not been demonstrated that the epididymis produces any substance which induces these changes.

CONCLUSIONS

1. Attempts to confirm the suggestion that spermatozoa attain their full maturity and are strengthened in consequence of some action of the epididymal secretion have been unsuccessful. On the contrary, evidence obtained from recent experiments indicates that the strengthening of sperm begins before they leave the testis and continues after they have been carried into the epididymis, but independently of any specific action of its secretion.

2. This theory does not minimize the importance of the epididymal secretion as a medium in which sperm are preserved during the maturing or ripening process. It would simply exclude the theory that the stimulus which induces ripening changes originates in the epididymis.

3. Until more attention has been devoted to the problem of the function of the epididymis, a tentative and probably incomplete hypothesis of the relationship between the epididymis and its contents, the spermatozoa, is given. It is re-emphasized that the epididymis is essentially a reservoir for sperm. In it the processes of sperm development which start while the sperm are still a part of the germinal epithelium are free to continue because of the favorable environment present in the organ.

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SEX DIMORPHISM IN THE SYRINX OF THE FOWL¹

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TWO PLATES (TWO FIGURES)

AUTHOR'S ABSTRACT

This study concerns the voice box or syrinx of the fowl in its possible relation to sex. Although neither hens nor capons crow, ovariectomized hens have been known to do so. It appears that a slight sexual dimorphism exists in the syrinx of certain fowls, and it was thought that a particular form might be essential to the act of crowing. However, no sexual dimorphism is apparent in the syrinx of the Brown Leghorn fowl.

The syringeal structures of crowing birds (cocks and ovariectomized females) contain no features which cannot be demonstrated in normal females. Variations were found in the syrinx in this breed and in other breeds, but they had nothing to do with sex. There is a gradual ossification with increasing age and size. It may be concluded that there is no apparent reason why the female fowl should not crow, provided it had the instinct to do so properly developed. The sex hormones, if they act in voice production, must act entirely through the conditioning of the central nervous system.

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INTRODUCTION

From the studies of Myers ('17) it appears that a slight sexual dimorphism exists in the voice box or syrinx of fowls.³ Such a dimorphism is of interest to those who are making a study of the secondary sexual characters. The Brown Leg-

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³Breed and age not specified.

horn fowl is one of the more strikingly dimorphic breeds the sexual characters of which are being studied to-day (Lillie, Domm, '27). When the females of this breed are ovariectomized on the left side, they grow testis-like glands on the right side and take on many of the male secondary sexual characters, including male behavior. Many of them have been known to crow (Domm, '27). Is this crowing a question of conditioning the nervous system by hormones, or is the male voice produced, as Myers' work suggests, only when the syrinx has a correlated structure distinct from that found in the normal female?

As a basis for discussion, a brief description of the syrinx may be given (see also Bronn, Myers, and Oppel). The rudimentary upper larynx is incapable of producing sound (see especially Oppel), and it has been demonstrated that the production of voice is limited to the syrinx (Myers). The syrinx of the fowl is of the 'tracheo-bronchialis' type, and lies, accordingly, at the tracheal bifurcation. At this level the respiratory tube is somewhat narrow. The syrinx proper includes all that lies between the first few rings at the lower end of the trachea and the first few half-rings at the upper end of each bronchus. Within these limits the most peculiar skeletal element is the cross-piece or pessulus. The pessulus extends like a prism across the inside of the respiratory tube, dividing the bronchi and, especially at its ventral extremity, providing attachment for other skeletal elements (Myers, fig. 4). The lower tracheal or 'tympanic' rings are the most anterior elements of the syringeal skeleton. They are not continuous with the pessulus. Just posterior to the tympanic rings are several rudimentary bar-like cartilages, the so-called intermediates, lying in the upper part of each side wall and joining the pessulus ventrally. Finally, below the pessulus there are cartilaginous half-rings on the lateral sides of each bronchus. The exact relation of each element to the others may be seen in Myers' figure 4. It will be noted that large openings are left in the syringeal skeleton laterally between the intermediate and bronchial cartilages and also

medially between the unclosed half-rings of each bronchus. It is in these openings that the real voice-producing elements, the vibrating or tympanic membranes, are located. The external tympanic membrane occupies the spaces in the lateral walls of the respiratory tube between the intermediate and bronchial cartilages, and the internal tympanic membrane occupies the medioposterior bronchial walls (Myers). The vibrating membranes and their skeletal supports are the parts of the syrinx mainly pertinent to the immediate discussion, but we should mention also certain extrinsic muscles, the sternotracheales and their additions, which manipulate the trachea and the syrinx as a whole. These muscles are attached to the tracheal rings above the syrinx.

In regard to dimorphism Myers says:

The main sexual difference to be noted in the syrinx of chickens is one of size. Male birds are usually larger than females, and so some differences would naturally be expected in the size of their voice organs. But this difference in the size of individuals is not sufficient to account for all of the differences observed. Quite naturally the sterno-tracheal muscles are smaller in females; but in the male the bony rings above the tympanum are entirely different in size and shape from the corresponding rings in the female. Again, in the male the tympanum is composed of the first four tracheal rings, while in the female only the first three form this structure. The tympanic membranes, however, exhibit no marked difference.

More concisely stated, the dimorphism which Myers observed in the vocal apparatus of the fowl appeared in the size of the syrinx as a whole, in the size of the sternotracheal muscles, and in the size, shape, number, and proximity of the rings in and above the tympanum. These last points may be understood better from Myers' figures 5 and 6, showing coronal sections. The supratympanic and tympanic rings of the male are shown as larger and squarer in section as the syrinx is approached, being set closer and closer together in the thick wall. In the female, on the contrary, the corresponding elements are smaller, rounded, and less numerous, appearing as outward swellings in the thin wall. The rings may be cartilage or bone—which suggests that there might

be a sexual distinction in their constitution. Finally, a sexual difference is stated to occur in the limits of the tympanum, which appear at the fourth ring in the male and at the third in the female.

In addition to pointing out these differences between male and female fowls, Myers says, "Sexual differences are very marked in song birds, especially as regards the size of the labia and the (intrinsic) syringeal muscles." These features cannot be demonstrated in the fowl. The external and internal labia, which occur in connection with the tympanic membranes, are hardly visible in the chicken, and the intrinsic syringeal muscles are entirely absent (Myers). The fact that the intrinsic muscles are absent in crowing fowls although they are always present in song birds might be considered as an indication that special structures are not needed for crowing.

Further support might be found for this idea, for Myers is of the opinion, in spite of the dimorphic findings, that "there is no apparent reason why the female should not be able to crow provided the instinct for it were properly developed." Myers' opinion is based on his experiments on the function of the syrinx. One experiment consisted of blowing air, by way of the cut end of a humerus, through the undisturbed air sacs and syrinx. I repeated this on a young (three-month-old) female and found that, by varying the flow of air and manipulating the body, sounds could be produced over a much wider range of tones than any normal hen or rooster employs.

It was suggested by Dr. Frank R. Lillie that I make a study of the syrinx in its possible relation to sex in the Brown Leghorn fowl. As was stated above, the ovariectomized female of this breed has been known to crow. The demonstration of a structural modification of the syrinx in the ovariectomized female would not be conclusive proof, of course, that that modification is indispensable for crowing, but the absence of such a modification would be a clear proof that it is not needed. The results of this study have been more conclusive

than we anticipated. In the Brown Leghorn fowl there is no sexual dimorphism of the syrinx, and there is no modification of the syrinx following either ovariectomy or castration.

MATERIAL AND METHODS

The material for this study has been made available by the kind cooperation of Doctor Domm, Doctor Juhn, and Doctor Mitchell, of this laboratory. Over one hundred specimens have been at my disposal, but unfortunately many of them have proved to be too young for the demonstration of the points at issue. The extremes of sexual dimorphism would be apparent only in adult birds, and certainly only extreme cases should be considered when the issue concerns the existence of a dimorphism which is slight at best. If the material is limited to birds over six months of age, it will include:

Sixteen normal males (fourteen at six to eight months, one of eleven months, one of twenty-one months); fourteen normal females, thirteen aged six to eight months, and one aged one hundred months (L499); nine capons, aged eleven to twenty-four months; fourteen ovariectomized females with right regenerated gonads, thirteen ranging from six to thirteen months, and one of seventy months (L503); three ovariectomized females with testis grafts, aged nine to ten months; one ovariectomized female with no regenerated gonad and no graft, aged nine months.

Of the ovariectomized females, two had actually been known to crow and six or eight others may have developed that ability without its being marked. For comparative purposes four old birds from other breeds were added to the list. They included one Silver Sebright male, one Golden Sebright female, one Plymouth Rock with a pathological ovary, and one Buff Orpington with a pathological ovary.

The study has been pursued by staining the entire respiratory tract from the upper larynx to the lungs and by staining coronal sections of the syringeal region, according to the various methods of Myers. All of the points at issue can be

demonstrated from midcoronal sections cut $20\ \mu$ in thickness and stained with Mallory's triple stain.

RESULTS

The syrinx of each Brown Leghorn fowl studied so far is approximately of the type described by Myers as specific for the male fowl. No sexual dimorphism has been revealed. The possibilities may be discussed, however, with regard to the sexual differences described by Myers.

1. The general size of the syrinx

Myers has designated size as "the main sexual difference to be noted in the syrinx of chickens." He thinks the size differences are so great that they cannot be accounted for wholly on the basis that male birds are larger than female birds, although he agrees to the fact and willingly ascribes part of the syringeal-size variation to it.

Males are indeed larger than females, as the figures given by the American Poultry Association show. The 'standard' body weight for Leghorn cocks is $5\frac{1}{2}$ pounds (2575 grams) and for hens, 4 pounds (1800 grams). An equal size difference was demonstrated here by Doctor Juhn and Doctor Mitchell. Of seventeen males aged eight to ten months the average weight was 1101 grams, and of thirteen females aged nine to ten months the average weight was 841 grams. From both sets of figures it would seem that males are about one-third larger than females. Most laboratory birds and certainly most of the birds used in the study of the syrinx are far below the American Standard in body weight, but, since fowls begin to crow long before they have attained full size, the absolute size is irrelevant and only the relative size is important.

It should be noted that Myers' figures do not indicate any size differences in the male and female syringes, except in the thickness of the tympanic walls and cartilages. Although it is hard to imagine that such slight size differences govern the act of crowing, they will be considered presently. But

just how great must the sexual difference in size be before it is significant?

This question can be answered by determining just how much of a general size variation there is in the syrinx. Size differences in the syrinx are very hard to demonstrate. The syrinx is a very elastic organ which is being stretched or retracted continually. Myers says, "The sterno-tracheal muscles by their contraction shorten the trachea and . . . also draw the tympanum cephalad, thus indirectly varying the tenseness of the tympanic membranes." Changes of tension occur during the act of crowing, for at that time roosters extend and retract their necks in a characteristic way, and, if they are placed in cages which hinder the extension of their necks, they squat down while crowing.

Measurements were made, nevertheless, on fixed and preserved material. Unless such material is deliberately stretched during its preparation, it is always shrunk and distorted, but we might assume a comparable shrinkage in all cases. On that basis measurements were made across the syrinx along various diameters and at various levels, and also along such distances as that from the first bronchial to the second tympanic ring. Such measurements were made with an ocular micrometer. Again, macroscopic measurements were made of the length of the pessulus and of the circumference of the tympanum by using calipers and thread. The more reliable measurements are tabulated in table 1, along with the age, body weight, and sex in each case.

Within each sex the measurements are highly variable. This may be due in part to the inaccuracies of the measurement and in part to the age and size variations of the birds. But it is clear that no well-marked size difference appears between males and females, and it is plain that such a sexual dimorphism would be demonstrable only in extensive statistics.

TABLE 1

SEX	CASE	AGE	WEIGHT	PESSULUS, LENGTH	TYMPANUM, CIRCUMFERENCE	DISTANCE, FIRST BRONCHIAL TO SECOND TYMPANIC RING
		<i>days</i>	<i>grams</i>	<i>mm.</i>	<i>mm.</i>	<i>mm.</i>
♂	W43	312	1638	5.5	13.8	5.8
	W908	262	1465	6.2	14.4	6.0
	m112	229	1336	6.2	15.0	5.0
	B	197	1321	4.2	14.5	4.8
	m194	229	1308	6.4	16.0	5.0
	E	199	1174	4.5	12.0	5.0
	A	197	925	3.4	11.0	5.0
	m119	229	975	5.8	14.5	4.8
	578	160	900	4.0	12.0	5.0
	714	129	823	4.8	14.0	5.4
♀	m138	229	809	5.6	14.0	5.8
	m2	153	785	5.0	12.5	4.8
	m3	160	725	5.5	13.0	5.0
	582	140	675	4.0	15.5	5.0

2. The size of the soft parts of the syrinx

Since it is the muscles and the membranes which are the functional parts of the syrinx, size differences in them would be expected more generally than in the hard parts. But such differences would be hard to establish. Measurement of the muscles was not attempted, although observations were made in more than fifty cases. The muscles seemed to be approximately of the same size in males and females, being unusually large only in birds in which the respiratory tract and the bird as a whole were correspondingly large. Again, no size differences were found in the membranes or in the epithelia of the syrinx. Even if size differences were demonstrated, it would still remain to be proved that the differences were due to sex directly. Muscles fluctuate in size with use and disuse, and it is conceivable that the rooster uses its vocal organs more strenuously than the hen, or vice versa.

3. *The size, shape, number, and proximity of the skeletal elements of the syrinx*

The skeleton of the syrinx and trachea of the Brown Leghorn fowl is uniformly like that described by Myers for the male fowl (compare his figs. 4 and 5). Regardless of age and sexual condition, the tympanic and supratympanic bones and cartilages of Brown Leghorn fowls are large, close-set, and squarish. Among the females; ovariectomized females, and capons studied not a single case shows the sparse, rounded rings characteristic of Myers' female cases, nor in comparison with the normal Brown Leghorn male do the other cases show any general divergence from the male type. It is not to be expected that older females would show any reduction or dedifferentiation of the syringeal structure, yet in Myers' female cases the skeleton is much less massive.

A more detailed comparative study was undertaken. It was thought that some unobserved sexual differences might be brought out. An attempt was made to count the number of elements of each kind in the syringeal skeleton, and to determine their size, their constitution as bone or cartilage, and their general configuration and contiguity. This study has revealed a great variation between different specimens which has nothing to do with sex. Variations were found in the bronchial rings, in the intermediates, in the tympanic rings, in the rings above the tympanum, and in the pessulus. The variations are slight but fairly well marked, and they are not due to any treatment given the respiratory tube during its fixation. The bronchial half-rings are the most changeable elements, perhaps. The bronchial half-rings may be set close together or widely separated; they may rest inside the bronchial walls or they may bulge out from them. The tracheal rings, both in the tympanic region and above it, are always set close together, but they are sometimes contiguous and sometimes not, sometimes fused and more often not. The intermediate cartilages may be large or quite the reverse. The pessulus may be cartilage, or bone, or both.

No doubt some of the cases studied are too young to show complete development, and some of the individual differences might disappear at older ages. For example, the trachea and syrinx gradually ossify, and this process provides a visible indication of growth in its later stages. More than fifty specimens of the respiratory tube, including some under six months of age, were stained with a specific cartilage stain— $\frac{1}{4}$ per cent methylene blue in 70 per cent alcohol with 1 per cent HCl added. This stain penetrated differentially and inversely to the degree of ossification as follows:

Ossification takes place first in the center of the pessulus and in the tracheal rings just above the tympanic cartilages. It proceeds gradually up the tracheal tube from ring to ring. In the syrinx the order to ossification is upset. The lower tympanic rings, the intermediate bars, and the bronchial half-rings never become ossified. Now, methylene blue does not penetrate those parts which are ossified, so it is the upper tracheal rings and the tympanic syringeal elements which stain best. Macroscopically, the intermediate cartilages are very minute, and they stain visibly only in those cases which show the least degree of ossification. In specimens which show a gradation of color down the tracheal tube, the least-ossified rings are completely blue, those which are ossified partially appear as four quadrants or partial quadrants of blue, and those which are completely ossified appear colorless.

The cases stained in this way were tabulated according to body weight, age, and sex. A rather indefinite correspondence appeared between ossification and age; a more convincing correspondence appeared between ossification and body weight; and no correspondence appeared between ossification and sex (table 2). Four cases are appended; those four are comparable to each other in age and weight.

A more careful study of the possible relation of ossification to sex was afforded by the coronal sections. The pessulus and the rings above the tympanum were examined especially in twenty-five cases chosen to include six normal males, six normal females, six castrated males or capons, six ovari-ot-

TABLE 2
Growth, as indicated by the transition from cartilage to bone

STAIN	WEIGHT	CASE	AGE	SEX	SECTIONED
Group I: All skeletal parts stained well, including inter- mediate cartilages	640	583	160	Poularde	X
	675	554	160	Poularde	X
	675	582	140	Normal female	X
	710	137	166	Normal female	X
	725	534	160	Normal female	X
	725	M3	160	Normal female	
	785	M2	153	Normal female	
	795	543	160	Normal female	X
	823	714	129	Normal female	
	900	578	160	Normal female	
	900	M4	160	Normal female	X
	900	M1	153	Normal female	X
	925	1120	97	Poularde	
	925	A	197	Normal male	
	1056	1146	216	Poularde	X
	1062	990	317	Poularde	
	1110	529	303	Poularde with testis	X
	1174	E	199	Normal male	
	1230	N	182	Normal female	X
	1237	1173	188	Poularde	
	1241	937	305	Poularde	X
	1276	1158	132	Poularde	
	1282	1121	141	Poularde	X
Group II: Cases grade from those having all but the intermedi- ate cartilages stained to those having only the bronchial, the first tympanic, and the upper tracheal rings stained	1286	1149	150	Poularde	
	1321	B	197	Normal male	
	1330	935	305	Poularde	
	1331	1129	227	Poularde	X
	1347	1077	237	Poularde	X
	1363	969	319	Poularde	X
	1465	W908	262	Male with one testis only	
	1638	W43	312	Male with one testis only	
	1893	W158	648	Male with one testis only	
	2015	W157	649	Male with one testis only	
	2195	W166	601	Capon	X
	2212	D9	575	Male with one testis only	
	2240	W154	651	Capon	X
	2248	W622	339	Normal male	X
	2276	W162	647	Male with one testis only	
	2315	W517	565	Male with one testis only	
	2330	W139	646	Normal male	X
	2330	W159	647	Male with one testis only	
	2482	W161	650	Capon	X
	2510	W151	648	Male with one testis only	
Group II middle Group II top Group II top Group II middle	2651	D11	550	Male with one testis only	
	2765	W160	650	Capon	
	1336	M112	229	Normal male	X
	1308	M194	229	Normal male	X
	975	M119	229	Normal female	X
	809	M138	229	Normal female	X

omized females or poulardes, and one poularde with a testis graft. In all but six of these cases the parts named were bony, but the six exceptional cases with cartilaginous parts included three normal males and three normal females. These cases were all under 900 grams in body weight, yet there were

TABLE 3

SEX	BIRD	AGE	WEIGHT	RINGS ABOVE TYMPANUM	PESSULUS
Male	C	198	900-1200	Bone	Bone
	D	198	900-1200	Bone (and cartilage)	Cartilage
	W139	646	2330	Bone	Bone
	W622	329	1248	Bone	Bone
	712	185	797	Cartilage	Cartilage
	713	185	959	Cartilage	Cartilage
Capon	D17		2464	Bone	Bone
	W39	354	1858	Bone	Bone
	W154	651	2240	Bone	Bone
	W161	650	2482	Bone	Bone
	W166	601	2195	Bone	Bone
	W545	354	1610	Bone	Bone
Poul-Tes.	529	303	1100	Bone	Bone
Poularde	969	305	1241	Bone	Bone
	937	237	1347	Bone	Bone
	1077	141	1282	Bone (and cartilage)	Bone
	1121	227	1331	Bone	Bone
	1129	116	1236	Bone	Bone
Female	1146	153	900	Bone	Bone
	M4	160	900	Cartilage	Cartilage
	137	166	710	Bone	Bone
	534	160	725	Cartilage	Cartilage
	543	160	795	Cartilage	Cartilage

only nine birds of such a light weight among the twenty-five studied. It is clear that ossification follows growth and not sex. The twenty-five cases are compared in table 3.

It will be noted that no poulardes or capons have cartilage in the pessulus or in the rings above the tympanum, except possibly case 1077, which seems to be in a state of transition. The rate of ossification may possibly be different in such

birds. It is regrettable that there are no light-weight birds among them, but perhaps younger birds can be added to the series later.

Even in those cases which are set down in the table as 'bony,' ossification does not occur throughout the entire tympanum. The lower two or three tracheal rings are always cartilage. Ossification begins in the third or fourth ring usually, and in the second or fifth ring exceptionally. The specimens are young, and there is too much variability among them to be sure whether or not there is a sexual difference in this regard; but in a majority of cases ossification begins in the third ring in females and in the fourth in males. From the study of older specimens more crucial evidence would be available, but it seems unlikely that females could be distinguished from males in this regard.

Meanwhile, we are confronted with the question of the limits of the tympanum, for that seems to be intimately bound up with the question of the lower limits of ossification. Myers says there are four tracheal rings in the tympanum of the male and three in the female. In describing the male Myers states that "the first four tracheal rings are fused to form the tympanum," and again that these "are very closely related to each other. They are partially fused along their sides as well as firmly bound together by dense fibrous tissue. Their ventral and dorsal extremities are free, but the spaces between these extremities are very narrow. This arrangement gives a very strong wall to this portion of the trachea, and because of this specialization this portion is known as the tympanum." However, there is no such regular specialization in the Brown Leghorn. There is very little fusion between even the lower two or three rings. Certainly, there is no distinct limitation of the tympanum at the third or fourth ring, and from Myers' figures 5 and 6 it may be questioned that his cases have such a limitation either. Again, the degree of ossification is not a criterion of the tympanic limits, because the tympanum is composed of both cartilaginous and bony rings. Myers says, "The first two rings are

composed of hyaline cartilage throughout; the third and fourth are entirely of bone, but each is separated from the next by a small space which is bridged by a heavy band of fibrous tissue." Again, the epithelium lining the tympanum is not a delimiting structure, nor has any previous worker called it one. The limits of the tympanum seem to be rather arbitrary.

A survey of all the findings as to the structure of the Brown Leghorn syrinx creates the impression that growth processes continue in it throughout the growth of the bird, and leads to the conclusion that slight sexual differences might be demonstrable in very old birds. Nevertheless, this study has demonstrated that the syringeal structure is not modified by ovariectomy or by castration, and that the normal pullet possesses all of the structures necessary to the act of crowing. This study has included two thirteen-month-old ovariectomized females which had been known to crow (cases 923 and 935). All of the structures found in the syringes of these birds were seen in the syringeal structures of normal young pullets and cockerels and in one two-year-old cock, and no structures have been seen in any male syrinx which have not been demonstrable in the normal female syrinx. In short, no effective sexual dimorphism exists in the syrinx of the fowl, if function is a criterion of effectiveness.

DISCUSSION OF PREVIOUS LITERATURE

From the wide variation of the syrinx in other birds (Bronn, Thierreich, II, Tafel 50) it had been anticipated that different breeds of fowls might show specific differences in the syringeal structure. Several cases in the literature demonstrate such differences. Garrod ('79) and Temminck (1813) both worked on the syrinx of the jungle fowl *Gallus bankiva*, which is different, apparently, from that of any domestic fowl studied before or since their time. Wunderlich ('84) refers, as a comparative anatomist, to the differences in the structure of the syrinx in the domestic fowl, in *Gallus bankiva*, and in *Euplocomus* sp. He believed that

the intermediate cartilages in the syrinx of the domestic fowl are reduced tracheal rings. He does not mention the breed of domestic fowl which he studied, but his description and figures are different from Myers' and mine. He points out differences in the articulation of the pessulus with various cartilages; and he says without qualification that ossification begins in the fifth tracheal ring (age and body weight not given). Wunderlich mentions one expression of sexual dimorphism in the syrinx which either does not appear or is not distinctive in the Brown Leghorn. That is, the second bronchial half-ring of the male, unlike that of the female, articulates with a bridge from the first bronchial half-ring. In the Brown Leghorn this bridge seems to develop in both sexes, but rather late in the course of development. Myers also mentions this attachment, but says nothing about a sexual difference concerning it. These minor differences illustrate the necessity for taking into account the developmental variability of the syrinx.

Another comparative anatomist concerned with the syrinx was Valentin Häcker ('00), and he seems to have made some observations on the fowl. Evidently, he recognized a sexual dimorphism of much the same kind as the one described by Myers, and Oppel summarized his views very nicely by saying that the female voice organ in fowls is relatively smaller in volume, more delicate in musculature, more primitive in the form of the skeleton, and that it has smaller labia than the male. Häcker mentions the work of Sellheim ('98) on the sexual dimorphism of the upper larynx and of the syrinx in fowls. Sellheim says he found size differences in the upper laryngeal structures of cocks, capons, and hens; but it is not clear that these were related to sex. Perhaps the study of the size relationships in other skeletal parts would clarify the relation of size to sex.

Some further proof that the syringeal structure of the Brown Leghorn differs from that of other breeds was found in the study of the syringeal structures of a Buff Orpington, a Plymouth Rock, and a Silver and a Golden Sebright Ban-

tam. These birds showed mutually differing structures, and each was different from the structure found in Brown Leghorns. A hasty survey shows no sexual dimorphism in the White Leghorn syrinx, which is like that of the Brown Leghorn.

SUMMARY

No sexual dimorphism has become apparent in the syrinx of the Brown Leghorn fowl, and there is no modification of the syrinx following ovariectomy or castration. With Myers, it must be concluded that "there is no apparent reason why the female (Brown Leghorn) fowl should not crow provided it had the instinct to do so properly developed." That instinct is apparently developed following partial (sinistral) ovariectomy, for, although neither hens nor capons crow, poulardes have been known to do so. Two such cases (923 and 985) have been available for the present study, and in them the syringeal structures contained no features which could not be demonstrated in normal females. Dr. F. R. Lillie suggests that a logical conclusion may be drawn regarding the rôle of the sex hormones in voice production, namely, that the sex hormones act entirely through the conditioning of the central nervous system.

Much variation was found in the form of the syrinx, especially in the younger birds. The variations occur chiefly in the size of the syrinx and in the number, size, shape, degree of ossification, and proximity of the skeletal elements. These variations have nothing to do with sex. They are also demonstrable between breeds, for example, between Brown Leghorns, Buff Orpingtons, Silver Sebright and Golden Sebright Bantams, Plymouth Rocks, and such unspecified breeds as those studied by Wunderlich, Häcker, and Myers.

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DESCRIPTION OF FIGURES

Midecoronal sections of syringes of Brown Leghorn fowls (projection drawings, magnification $\times 5$).

In each figure the trachea is above and the bronchi are below. The tracheal walls are supported by rings of cartilage (stippled) or bone (solid black). The lower three or four tympanic rings, the so-called tympanics, are immediately above a narrow region to which much smaller cartilages, the intermediates, lend support. At this level the walls are vibratory; they are called external tympanic membranes. Farther posteriorly are the bronchi supported laterally by cartilaginous half-rings which do not extend to the inner walls. The inner walls of the bronchi are the internal tympanic membranes. In section they appear to be suspended from the cartilaginous or bony cross-piece, the pessulus, lying like a prism at the bifurcation. The pessulus is attached dorsally and ventrally to the intermediate syringeal cartilages in—or above, as you choose to define them—the external tympanic membranes.

PLATE 1

EXPLANATION OF FIGURE

Normal fowls. Top row, males. Bottom row, females.

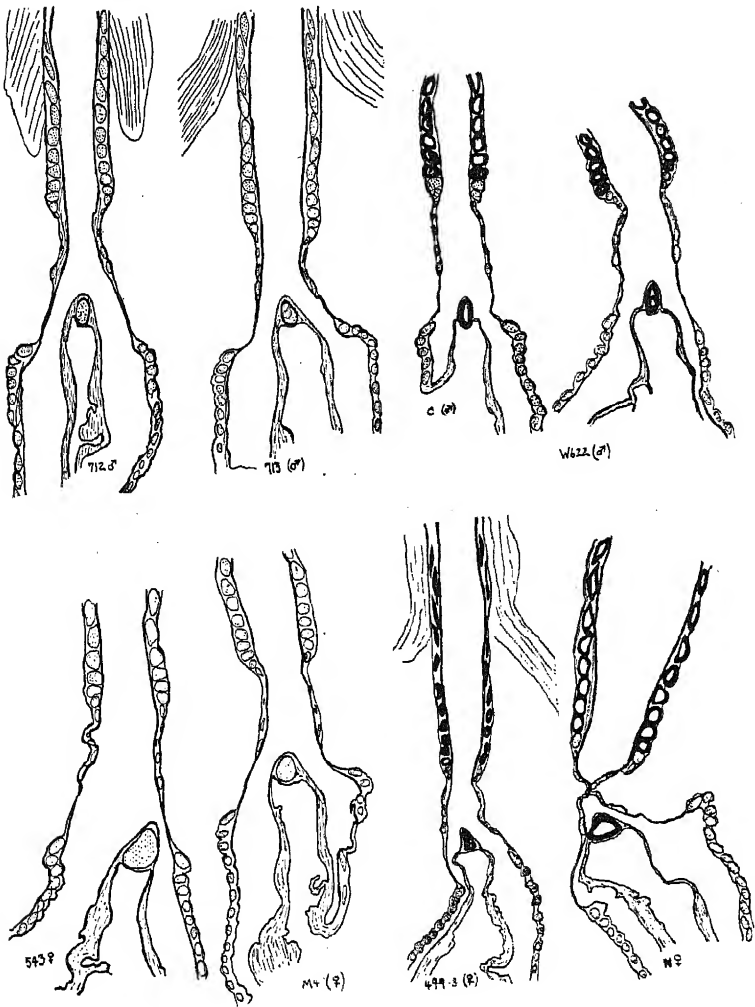
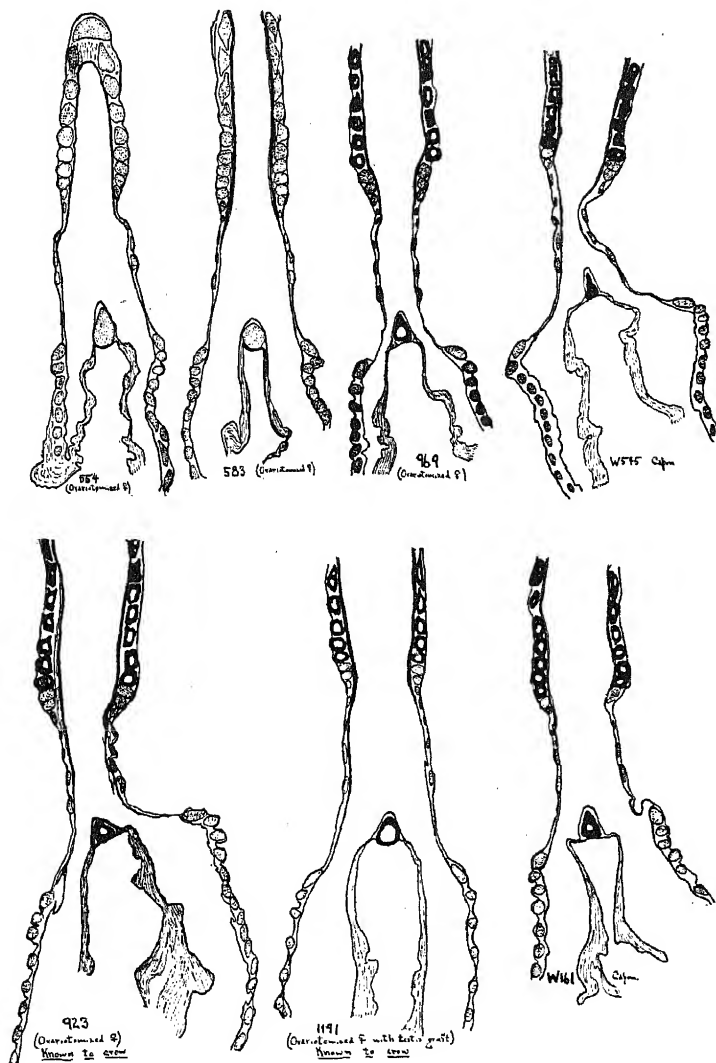


PLATE 2

EXPLANATION OF FIGURE

Experimental fowls. Top row, left to right, ovariectomized females 554, 583, and 969, and castrated male W545. Bottom row, left to right, ovariectomized females 923 and 1191, both known to crow (1191 had a testis graft), and castrated male W161.



THE ANATOMICAL QUALITIES OF THE LIVER DURING THE VARIOUS STAGES OF ITS FUNCTIONAL ACTIVITIES

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EIGHT FIGURES

AUTHOR'S ABSTRACT

The liver has a rhythmic function with alternating assimilatory and secretory stages. The height of the assimilatory stage in rabbit's liver is characterized by the following qualities: All the cells are usually expanded and contain much glycogen and few bile components. The bile capillaries are narrow and generally empty. The glycogen content is high (about 13 per cent); the total glycogen weight is also high (about 17 grams). The liver has a relatively heavy weight (about 140 grams), a large volume, notably firm consistency, and a light brown color.

The secretory stage at its height has the following characteristics: All cells are shrunken and are rich in bile components, but contain little glycogen. The bile capillaries are expanded and filled with secretion (fixation with barium chloride). The glycogen content is low (about 1 per cent), and the total glycogen weight is also low (about $\frac{1}{2}$ gram). Weight of liver low (about 50 grams). Volume small and consistency fairly flaccid. Color dark reddish brown.

Between these two extremes are various intermediary stages. Such variations as these probably occur during the normal functioning of the liver in man and also in other animals.

Acquaintance with the normal variations in the structure of an organ is a necessary prerequisite for the recognition of its pathological changes.

In hardly any other organ are the normal variations of such great significance as in the liver, the appearance of which varies widely in the different stages of its functional activities. In order to learn to recognize these stages, it is necessary to examine a very large number of normal livers under various conditions. I have had occasion to examine thoroughly the livers of 140 rabbits, both with the naked eye and under the microscope, and in several of these livers I have also made a quantitative determination of the glycogen content. Because of the common use of rabbits in experiments and because the different functional activities and the anatomical qualities accompanying them are probably similar in man and the rabbit, I feel justified in here briefly summarizing my results.

I have found that it is possible to distinguish between two opposing functional stages of the liver, viz., that of secretion and that of assimilation. When the secretory function is at its height, the liver has an abundant content of bile components in all the liver cells, but very little glycogen; when the assimilatory function is at its height, the liver cells contain much glycogen, but only a small quantity of bile components. Between these two extremes there are any number of intermediary stages.

At the height of the secretory function, the appearance of the liver may be described as follows:

The organ is strikingly small and light in weight; a liver weight of 50 grams has in several cases been found during this functional stage in adult rabbits weighing about 2 kg. The color is dark, brownish red, or red-black. The outlines of the lobules are indistinct, and the surface is smooth. The consistency is rather flabby, which is probably due to the low water and glycogen content, and the consequent decrease in turgor.

The glycogen content is sometimes as low as 1 per cent, which in a liver weighing 50 grams is only 0.5 gram in all.

Microscopic examination demonstrates the fact that the liver cells are exceedingly small, sometimes with a diameter of only 0.016 mm.

The bile capillaries are wide and filled with substances precipitated by barium, i.e., bile components, which are present in great quantities in the liver cells also (compare figs. 1, 2a). Glycogen is, on the contrary, extremely scarce; most of it is seen in some few cells nearest the central vein in each lobule (compare fig. 2b).

This extreme stage is of course relatively rarely obtained. Formerly, it was thought that so low a glycogen content as this occurs mainly after hunger or muscular exertion. I have, however, observed it in animals at rest after only five or six hours' lack of food, at which time the digestive tract still contains much nutriment. Even in entirely normal animals at rest and suffering from no deprivation of food have

I observed almost equally low glycogen values in the liver during its secretory functioning.

At the height of the assimilatory function, the appearance of the liver may be described as follows:

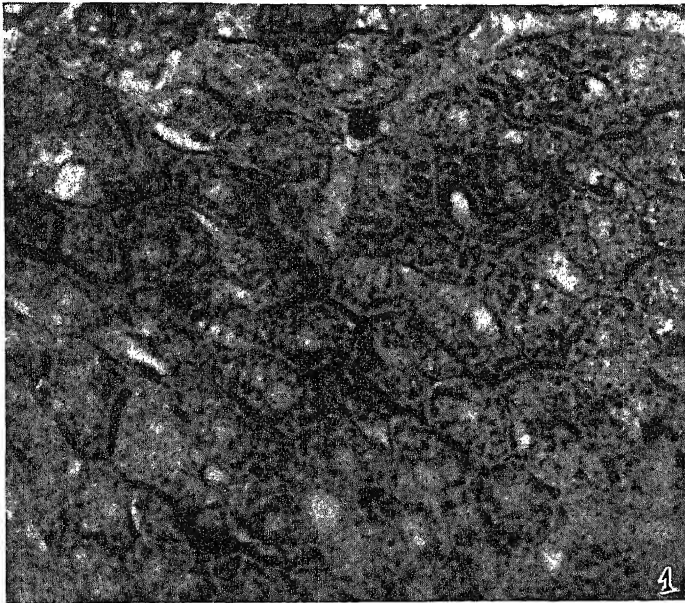


Fig. 1 Section of liver at height of secretory function (weight of liver, 50 grams; glycogen content, 1 per cent). Fixation in barium chloride and formalin; stained with acid fuchsin (Mallory).¹ Enlarged 610 times. Liver cells small (diameter about 0.016 mm.). Bile capillaries wide and filled with bile components precipitated by barium chloride. Cells also well supplied with bile components.

The organ is strikingly large and heavy. A weight of 144 grams has at this stage been observed in an adult rabbit weighing about 2 kg.

¹Note: Small pieces of liver should first be fixated for about six hours in a 3 per cent solution of barium chloride, then for eighteen hours in a 10 per cent solution of formalin; after this they should be rapidly soaked in alcohol, treated with benzol, and embedded in paraffin. The bile components precipitated by barium chloride can be stained with acid dyes, preferably acid fuchsin (Mallory's connective-tissue stain).

The color of the liver is light brown, often slightly watery.

Sometimes the surface shows a fine granulation, which is probably due to the fact that the lobules bulge out because the liver cells are stuffed with assimilated components. This granulation should be distinguished from that in cirrhosis, in which it is caused by the shrinking of the connective tissue.

The liver is on palpation felt to be very firm and taut.

The glycogen content is high (up to 13 per cent). A total glycogen content of as much as 17 grams has been observed.

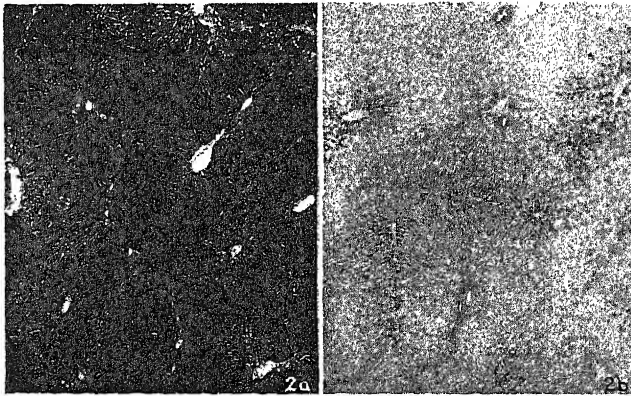


Fig. 2 General view of the liver of figure 1. Enlarged twenty times. a. After fixation with barium chloride; all the liver cells well supplied with bile components. b. After fixation with alcohol and glycogen staining (Best); very little glycogen, mainly in cells around the central veins.

Under the microscope, the liver cells are found to be conspicuously large, with diameters of up to about 0.036 mm. (compare fig. 3).

The bile capillaries are narrow and shrunken, containing only very little or no substance precipitated by barium (bile components) (compare fig. 3). The liver cells, too, contain only very little or no bile components (compare figs. 3 and 4a). Glycogen, on the other hand, is amply present in all the cells (compare fig. 4b). When the rabbits have been given food that is rich in proteins, large clumps of albumen deposits can be seen in the liver cells (compare fig. 5).

This extreme stage of the assimilatory function is naturally also more rarely obtained. In my experiments I found it most frequently at about 2 o'clock in the morning, or between 2 and 4 of the afternoon, but it may occur at other times also.

The most common state of the liver is, of course, somewhere between these two extremes. Such intermediary stages may vary in appearance, thus:

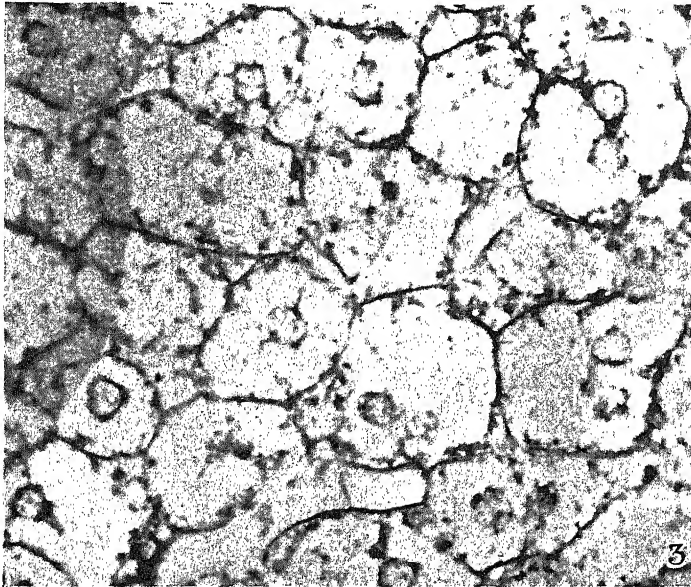


Fig. 3 Section of liver at height of assimilatory stage (weight of liver, 133 grams; glycogen content, 12.9 per cent). Fixation and staining as in figure 1. Enlarged 610 times. Liver cells large (diameter about 0.036 mm.). Bile capillaries narrow or collapsed, mostly empty. In the liver cells, a fine net of protoplasm, but a very small quantity of bile components. (Compare with fig. 1, and observe the great difference.)

Immediately after the stage of most intense assimilation follows one in which particles of secretion begin to appear in those liver cells which are situated in the periphery of the lobules; the cells, nevertheless, do not lose their glycogen content. Little by little, the droplets of secretion appear in all the liver cells, which now contain a moderate quantity of

both glycogen and bile components. Then the bile components in the peripheral cells of the lobules increase in quantity, while the glycogen in them disappears. This condition is

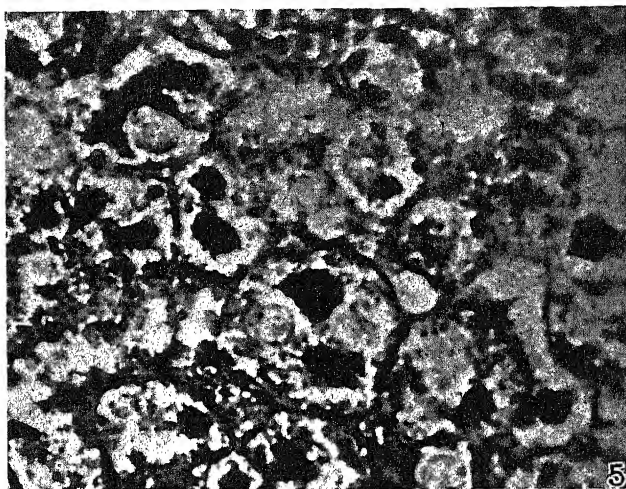
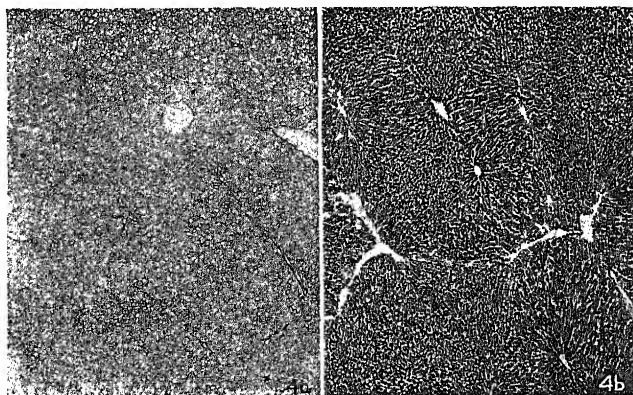


Fig. 4 General view of the liver of figure 3. Enlarged twenty times. a. After fixation with barium chloride; all the cells pale because of the scarcity of stainable substances, i.e., bile components precipitated by barium chloride. b. After fixation with alcohol and glycogen staining (Best); all the cells abundantly supplied with glycogen.

Fig. 5 Section of liver after a diet rich in proteins. In the cells, large irregular clumps of albumen. Otherwise same as figure 3.

illustrated by figures 6 and 7, which give examples of a division of labor in the lobules, in that the cells in their periphery are in the secretory stage, while those in the interior are in the assimilatory stage.

This circumstance can be observed macroscopically also; the periphery of the lobules looks a little darker than the interior. This appearance is not to be confused with the similar one due to the uneven distribution of blood which may exist in pathological cases. Such an uneven distribution is never found in animals which have, like those under discussion here, been killed by decapitation, after which the entire liver is deficient in blood.

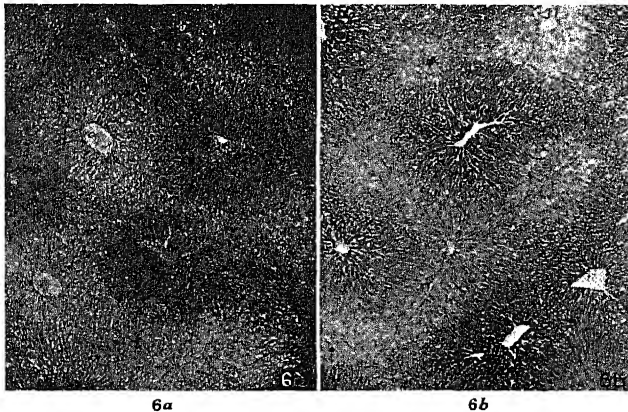


Fig. 6 General view of liver in intermediary stage (weight of liver, 86 grams; glycogen content, 4 per cent). Enlarged twenty times. a. After fixation with barium chloride; in the periphery of the lobules the liver cells are well supplied with bile components, while the cells of the interior of the lobules are scantily supplied. b. After fixation with alcohol and glycogen staining (Best); much glycogen in interior of lobules, little in the periphery.

When the zone in which the liver cells are in the secretory stage has spread until it includes the whole interior all the way to the central vein, the height of the secretory function as described above will have been reached.

During these intermediary stages, the glycogen content and the weight of the organ gradually diminish. The process is illustrated by the diagram (fig. 8).

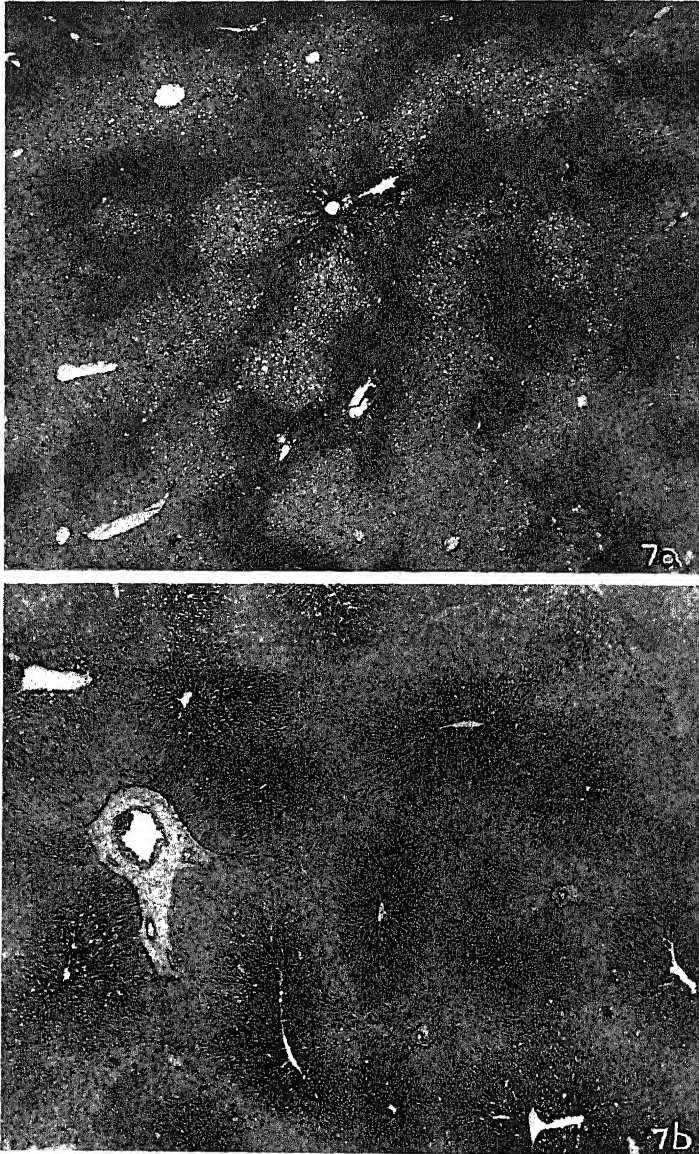


Fig. 7 a. General view of a liver in which peripheral cells of the lobules are amply supplied with bile components. Enlarged eighteen times. Fixation with barium chloride. b. After fixation with alcohol and staining of the glycogen; much glycogen in interior of lobules, little or nothing in the periphery.

The process described above is reversible. When the deposition of glycogen, which begins in the interior of the lobule, advances toward the periphery, we finally obtain again the stage of most intense assimilation described above. The simultaneous great increase in weight is most probably due to an increase of the water content.

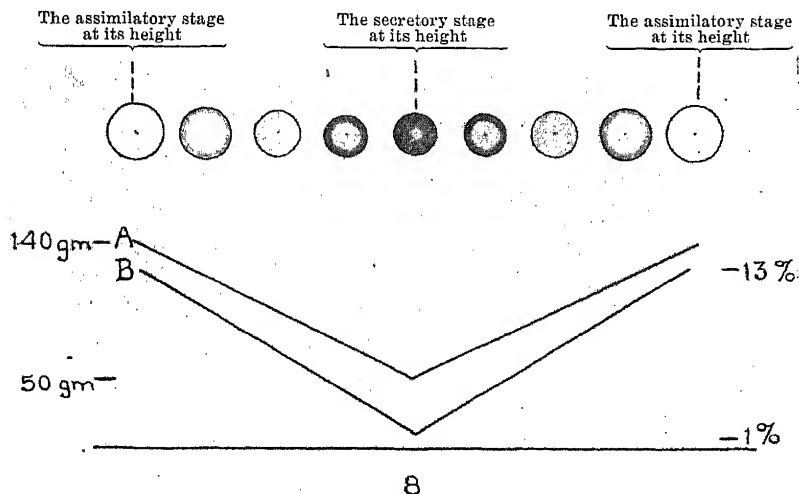


Fig. 8 Diagram of the functional stages of the liver. The circles represent the lobules of the liver with vena centralis in the center. The white areas represent cells that are rich in glycogen, but deficient in bile components; the black ones, those that are rich in bile components, but deficient in glycogen; the gray ones, those containing both bile components and glycogen. A is the weight curve, and B, the glycogen curve during the functional stages.

The cells in the periphery of the lobule, in which the production of bile begins first and ceases last, are more secretory in character, while those in the interior, in which the production of glycogen begins first and ceases last, are more assimilatory in character. This circumstance has probably some relation to the anatomical structure of the liver; the peripheral cells in the lobules are most likely the youngest and most active, and are best supplied with arterial blood. The bile-capillary system is also more developed in the periphery. These circumstances are undoubtedly of great

importance for the understanding of the localization within the lobules of the liver of different pathological processes.

In adult rabbits weighing about 2 kg., the liver has an average weight of a little less than 100 grams. The average glycogen content is 7.5 per cent. The normal variations are, nevertheless, very great, as we have seen from the above; it follows that it is very difficult to know whether an increase or decrease in the size of the liver is pathological or not.

One may be tempted to think that weighing the liver is a reliable method of discovering whether there is hypertrophy or atrophy. But it is also necessary to take into account the functional stage of the liver and, most particularly, its glycogen content.

For, as we have seen from the above, the liver may at the height of assimilation have more than double the weight it has at the height of secretion, since, like a sponge, it takes up more than its own weight of substances resorbed from the intestine. To use a slightly exaggerated simile, determining the weight of the liver without taking into account the weight of the assimilated substances is like determining the weight of the ventricle without considering the weight of its contents.

Similar difficulties arise in attempting to tell whether an increase or decrease in the glycogen content of the liver is pathological or not. Normally, it may vary from 1 per cent to 13 per cent, or from about 0.5 gram to 17 grams. Many authors who have found low glycogen values in the liver believed that the condition was due to artificial factors, such as hunger, muscular exertion, extirpation of the pancreas, injections of adrenalin, administration of insulin, etc. It is quite possible that the low glycogen values in many of these cases were normal, and due to the fact that the liver happened to be at the height of the secretory stage at the time, as it occasionally is under entirely normal conditions.

It is important to emphasize the necessity of having long series for control in studying the glycogen content of the liver in animal experiments. This requirement has in a large number of investigations been disregarded.

As mentioned above, the color and consistency of the liver also vary during its different functional stages.

My conclusion is thus briefly:

In order to determine to what extent the anatomical qualities of a liver are pathological, it is necessary to be thoroughly acquainted with the normal variations in liver structure in connection with the various stages of the liver functions. The formation of a reliable opinion presupposes taking into account in each individual case the functional stage in which that case happened to be at the time. This must also be considered in judging animal experiments.

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THE STRUCTURE AND CHROMOSOMES OF THREE GYNANDROMORPHIC KATYDIDS (AMBLYCORYPHA)

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THREE PLATES (TWENTY-EIGHT FIGURES)

AUTHOR'S ABSTRACT

The external genitalia and the internal reproductive organs are described. Compound gonads, with a normal development of testicular and ovarian tissues, are found in the positions of the gonads of normal individuals. The genital ducts arise in both types of tissue and serve as common ducts.

It is demonstrated microscopically that the male tissue has one and the female tissue two sex chromosomes. The diploid number (or nearly so) is present in both tissues. This is the constitution of the normal sexes.

The origin of the gynandromorphs is accounted for by assuming either the theory of dispermy in the binucleated egg or chromosomal elimination.

INTRODUCTION

Among the insects, gynandromorphs have offered a favorable opportunity to study the influence of the sex chromosomes on the production of sex. Many abnormal forms have been described and figured, and the extensive genetic studies of various workers, particularly Morgan and Bridges ('19), Sturtevant ('20), and Whiting and Whiting ('27), have brought to light many hybrid gynandromorphs whose genetic constitutions were known. In these forms the genetic constitution has frequently revealed the chromosomal composition of the male and female tissues, but a successful microscopical examination of the chromosomes has never been made in both male and female tissues. Usually, this has been due to the fact that characteristics by which an abnormality could be detected during the growing nymphal stage when the cells are undergoing rapid mitotic divisions are absent, or that the chromosomes have not been favorable for a microscopical study.

Kornhauser ('19) described a gynandromorph of the membracid *Thelia bimaculata* (Fabr.) which was externally a normal-appearing female, but a pair of testes was found in the abdomen instead of ovaries. The testes contained typical cysts and spermatogonia with the normal male number of chromosomes (twenty-one). No metaphase plates from the soma cells were clear enough to count, but two good pro-phases indicated the presence of the normal female number (twenty-two). No statement was made as to whether two sex chromosomes could be distinguished and no figures of these pro-phases were given. However, two sex chromosomes were assumed to be present.

The sex chromosomes were distinguished in both male and female tissues in two of the gynandromorphs described in this paper.

The author expresses his appreciation for the aid of the following men: Dr. Edmund B. Wilson made many helpful suggestions during the progress of the study at Columbia University. Dr. Fernandus Payne, of Indiana University, suggested the problem which led to the discovery of the gynandromorphs. Dr. Will Scott, Director of the Indiana University Biological Station, Winona Lake, Indiana, granted research facilities during the summers of 1926 and 1927. Mr. James A. G. Rehn, of the Academy of Natural Sciences of Philadelphia, identified the adult specimens.

MATERIAL

The specimens were collected during the summer of 1920 near the Indiana University Biological Station, Winona Lake, Indiana, but the study was not undertaken until 1926. An unsuccessful search for more gynandromorphic material was made at the place where the original specimens were taken during the summers of 1926 and 1927, and elsewhere during 1928.

Two of the gynandromorphs were detected during the nymphal period by their coloration.

The normal sexes differ slightly in general coloration, but there are no other color markings which distinguish them during the nymphal periods. Normal males may be described as yellowish green, whereas the normal females lack the yellowish tinge and may be described as green. When searching for normal nymphal males to be used in another problem, the males were chosen more readily by observing the yellowish-green color than by observing the external genitalia.

The first gynandromorph was mistaken for a normal male because it had a yellowish-green color. It was recognized to be abnormal when the genitalia were observed to resemble an ovipositor.

The second gynandromorph was distinguished in the field by its yellowish-green color alone.

The third gynandromorph was not recognized as an abnormal individual until the gonads were sectioned for study. It was collected at the same time, from the same clump of nettles, and belonged to the same species as the second gynandromorph. These facts suggested that the two may have belonged to the same brood.

Less than 500 individuals had been examined at the time the last gynandromorphs were found.

The taxonomic characters which are used to determine the species of *Amblycorypha* are absent during the nymphal periods; consequently, it was necessary to determine the species to which the gynandromorphs belonged by comparing the chromosomes of the gynandromorphs with those from adults whose species had been determined. As a further aid in the determination, normal nymphs were reared under observation and compared with the preserved gynandromorphs.

The gynandromorphs will be referred to in the order in which they were collected as gynandromorphs A, B, and C.

Gynandromorph A represents *Amblycorypha rotundifolia rotundifolia* (Scudder); gynandromorphs B and C, *Amblycorypha oblongifolia* (DeGeer).

TECHNIQUE

Gynandromorph A was killed in ether; B and C were dissected alive. The normal specimens were killed in either ether or xylol, or dissected alive. All gonads were removed in normal salt solution.

Two fixing reagents were used. The gonads of gynandromorph A were fixed for eighteen hours in a modification of Bouin's solution which was made of 75 cc. of saturated aqueous picric acid, 20 cc. of formalin, 5 cc. of glacial acetic acid, 2½ grams of urea crystals, and 2 grams of chromic acid. The gonads of gynandromorphs B and C were fixed for twenty hours in Flemming's strong solution. The material used for comparison was fixed in both solutions; the time was varied from two to twenty-four hours. The modified Bouin's fluid, used for the shorter periods, gave the best results for the chromosomes, but the achromatic figure was rather poorly represented.

The gynandromorphic material was stained in Heidenhain's iron-haematoxylin. The material used for comparison was similarly stained, with the addition of eosin to some of the slides as a counterstain.

After dissection, all of the nymphs and some of the adults were preserved in 85 per cent alcohol. The other adults were dried.

THE REPRODUCTIVE ORGANS

Normal individuals

The structure of the reproductive organs of normal individuals was examined in the nymphal and adult stages. Particular attention was given to the fourth instar, because gynandromorph A, the most valuable specimen for the study, was taken in that instar.

Female. Adult katydids belonging to the genus *Amblycorypha*, in common with several other tettigoniids, possess characteristically large, serrated, and saber-shaped ovipositors, which consist of paired dorsal, ventral, and inner valves (figs. 1 and 2). The dorsal and ventral valves fit closely

together, forming the external portions of the organ, while the inner valves fit together internal to the upper valves. At the time of hatching, the ovipositor is represented by thickenings on the ventral side of the eighth and ninth segments. The dorsal and inner valves develop from a distinct pair of papillae that are located on the ventral surface of the ninth segment, while the lower valves develop from a pair of thickenings which appear on the eighth segment. With successive ecdyses the valves increase in size and length. In the fourth instar they are strong and fairly well developed, but the serrations which are characteristic of the adult organ appear later.

The internal reproductive organs of the adult consist of paired ovaries and oviducts, a seminal receptacle, and a vagina (fig. 8). Although immature during the fourth instar, they are well developed. The ovaries are formed of compact tubules, lying side by side, which converge anteriorly into a terminal filament that is attached to the body wall dorso-laterally. Immediately posterior to the terminal filament are the oogonia. These are followed by oocytes in successive stages of the growth period. Soon after entering the growth period, the oocytes are separated by follicular epithelium. The ducts from the tubules are elongated slightly before converging to enter the oviduct, which leads posteriorly into the vagina. The seminal receptacle lies dorsal to the vagina. Its duct is thrown into a single loop before entering the vagina from above.

Male. The subgenital plate and aedeagus of the fourth instar, although immature, distinctly indicate the adult structures (figs. 6, 7, 10, and 11). The subgenital plate develops from the ventral surface of the ninth segment. A pair of movable spines tip the ends of the notched subgenital plate.

The eighth segment of the male does not give rise to any part of the external reproductive organs, and in this respect differs from the female, where the eighth segment gives rise to the paired valves of the ovipositor.

The internal reproductive organs of the adult consist of paired testes, vasa deferentia, seminal vesicles, and primary and secondary accessory glands. These organs are present in the fourth instar, with the exception of the primary and secondary accessory glands (figs. 10 and 11). These glands are represented during the fourth instar by the thickened walls of two pairs of vesicles which develop during the preceding stages. During the final nymphal instar and the early imago the smaller pair of these vesicles gives rise to the primary accessory glands and their ducts by outgrowths from the thickened walls, whereas the larger pair gives rise to the secondary accessory glands and ducts, and to the seminal vesicles.

The testes consist of a mass of elongated tubules whose inner ends are directed toward a centrally situated duct—thus giving the general appearance of a bunch of grapes. The centrally situated duct emerges from the testis as the coiled epididymis. It is somewhat smaller in the lateral extent of its convolutions than the diameter of the oviduct (figs. 8 and 10). This size relationship is retained throughout development and in the adult stage. The epididymis is not readily distinguished by a superficial examination as a convoluted duct until the final instar, but when it is removed from the earlier instars and sectioned it is seen to be distinctly convoluted (fig. 16). Posterior to the epididymis the vasa deferentia converge to enter the seminal vesicles.

Description of gynandromorph A

Collected July 11, 1920. Late fourth instar. The color was observed to be like that of the male. The eighth tergite was separated into two parts by a division of the lower left side (figs. 3 and 5).

External genitalia. A normal half of an ovipositor, with well-developed dorsal, ventral, and inner valves, was present on the right side; the left side possessed characteristics of both sexes (figs. 3, 4, and 5). On the latter side an abnormal plate had developed from the ninth segment in the place of

the normal dorsal and inner valves of the ovipositor. In shape the plate was somewhat shorter and much wider than a normal dorsal valve, and its attachment to the ninth segment was similar to half the attachment of a subgenital plate. In the preserved animal the broad inner surface of the abnormal plate fitted closely against the normal half of the ovipositor, similar to the position of the normal dorsal valve. A very thin membrane extended across and covered the inner surface of the abnormal plate. The position of this membrane indicated that it may have represented an imperfectly developed inner valve, since the dorsal and inner valves develop from the same embryonic outgrowth of the ninth segment.

A papilla was located on the left side of the eighth segment opposite to the base of the normal right lower valve (figs. 3 and 5). Its structure and position resembled the thickening which gives rise to a lower valve of the ovipositor in the normal individual. Since there are no outgrowths from the ventral surface of the eighth segment in the male, the papilla, evidently, represented a rudimentary lower valve of the ovipositor.

Internal reproductive organs. An ovotestis was present on each side. The compound gonads were unequal in size, the smaller being about two-thirds the size of the larger. About equal amounts of ovarian and testicular tissue were present. Both tissues were compact and appeared to be in a healthy condition (figs. 14 and 15). The developing oocytes were located in tubules which converged into a terminal filament. Successive stages in the process of oogenesis were present along the tubules as far as the middle growth period; here, the germinal vesicles were as large as those in the normal individuals and the cytoplasm contained an apparently normal amount of yolk. Some of the ovarian tubules were short, and consequently contained fewer oocytes.

Normal testicular cysts were present in which the various phases of spermatogenesis occurred to the beginning of the transformation of the spermatids.

A single genital duct led posteriorly from each ovotestis. Each duct had its origin in both ovarian and testicular tissue (figs. 14 and 15). As the common ducts emerged from the compound gonads their size was the same as normal oviducts, but posteriorly they constricted and approximated the size of the vasa deferentia (figs. 12 and 13). This constriction was not observed until a careful examination was made of the ends left attached to the posterior parts of the internal reproductive organs at the time the gonads were removed. Consequently, the ducts were considered as apparently normal oviducts in the preliminary report ('27).

The posterior parts of the internal reproductive system were very abnormal and distorted (figs. 12 and 13). Three abnormal vesicles of different sizes were present. The largest of these vesicles received the common genital ducts and probably represented the paired seminal vesicles, since these vesicles receive the vasa deferentia in the normal male (fig. 10). The second abnormal vesicle was considerably smaller and directly continuous with the side of the larger. It probably represented one of the paired vesicles which gives rise to the primary accessory glands. The third abnormal vesicle was connected with the other two by connective tissue only. Its position was dorsal to the entrance of the common ducts. It may have represented the seminal receptacle of the female. The first two described vesicles were directed posteriorly, the reverse of the normal position, so that the tip of the larger lay at the base of the valves of the ovipositor. The ventral side was twisted laterally so that the common ducts entered the rudimentary seminal vesicle somewhat from one side.

Neither vagina nor aedeagus was represented, and there was no opening leading from the abnormal vesicles for the escape of the genital products.

A gynandromorph with such abnormal reproductive organs could not function as either male or female during copulation. The eggs might be self-fertilized, provided a medium were present in the ducts through which the spermatozoa could migrate; but there would be no means for the escape of the

fertilized eggs from the body, except by the rupture of the abdomen. Such an individual would probably be unable to produce offspring.

Description of gynandromorph B

Collected July 25, 1920. Fifth instar. The color was observed to be like that of the male; otherwise it could not be distinguished from a female by an external examination.

External genitalia. Normally developed.

Internal reproductive organs. An ovary was present on the left side and an ovotestis on the right (fig. 9). The ovary was slightly reduced in size, but it was normal in other respects. The ovotestis contained about three times as much testicular as ovarian tissue. Most of the oocytes in the compound gonad were healthy and normal in appearance; a few, however, were degenerating. These were closely surrounded by healthy testicular tissue. The proximity of this testicular tissue and the apparent inability of the epithelial tissue immediately surrounding the eggs to supply nutriment for further growth and development seemed to be the cause for degeneration. The degeneration was not due to the inability of the animal to assimilate food. This fact was shown by the eggs which were developing normally in other tubules of the ovotestis and in the ovary. There was no evidence of degeneration in the male tissue.

The testicular cysts contained many stages of spermatogenesis and very nearly mature spermatozoa.

The ovarian and testicular tubules of the compound gonad opened into a centrally situated duct. This common duct emerged from the gonad as an apparently normal oviduct. The duct leading from the ovary was a normal oviduct. The posterior part of the reproductive system was normal.

Since all of the reproductive organs were like those of the normal female with the exception of the ovotestis, the gynandromorph could have functioned as a normal female in copulation and fertilization. Self-fertilization was also a possi-

bility by means of the common genital duct, provided a medium were present through which the spermatozoa could migrate.

Gynandromorph C

Fifth instar. The specimen was an apparently normal male, with the exception of the gonads. One of these contained a small ovarian tubule with oocytes which had entered the early part of the growth period. The tubule was connected to the same system of ducts which led from the testicular tubules. There were no cells in the female tissue in which chromosomes could be distinguished.

The other gonad contained several enlarged cells with very large nuclei. A few metaphase plates indicated a polyploid number of chromosomes, and this may have given rise to the enlarged nuclei.

The greater mass of the testes contained normal spermatogonial cysts.

CHROMOSOMES OF MALE TISSUE AND SPERMATOGENESIS

In the study of the chromosomes gynandromorph A was the most valuable, because of the presence of a great number of division figures. All of the figures of chromosomes which were drawn from gynandromorphic material were made from this specimen with the exception of figure 26, which was drawn from gynandromorph B.

The number of chromosomes in the male tissue of gynandromorph A was determined from a study of five spermatogonia and fifteen spermatocytes. All were ideal metaphase plates. The spermatogonia contained thirty-three chromosomes; the spermatocytes of the X class, seventeen, and those of the no-X class, sixteen. These chromosomes corresponded in number, size, and shape with normal specimens of *Amblycorypha rotundifolia rotundifolia* (Scudder). The gynandromorph agreed with this species morphologically.

There were fewer metaphase plates from the male tissues of gynandromorphs B and C. These plates showed that each specimen had thirty-five chromosomes. The chromosomes

corresponded with the chromosomes from normal specimens of *Amblycorypha oblongifolia* (DeGeer). Both gynandromorphs also agreed with this species morphologically.

The work of O. Mohr ('16) on the heterochromosomes of the European grasshopper, *Leptophyes punctatissima*, proved to be a very valuable aid in following spermatogenesis. The chromosomes (male and female diploid groups reproduced by Wilson, '25, fig. 355, C. D. p. 751) were very similar in form, size, and number to those of *Amblycorypha*, and the changes which occurred during the growth period and meiotic divisions were found to be the same. Consequently, no attempt will be made to describe the process in detail.

Many different stages of spermatogenesis were observed and compared with normal material. The process was normal for each gynandromorphic individual.

The sex chromosome is easily followed in the changes that take place during the growth period and the meiotic divisions (figs. 17 to 26). At the end of the final spermatogonial division the sex chromosome remains condensed and moves toward the periphery of the nucleus, where it stays, distinctly visible, throughout the growth period (fig. 21). The primary spermatocyte division is reductional for the sex chromosome. During the metaphase it may be found lying to the side of the equatorial plate, where it remains a short time only. In the succeeding anaphase it moves undivided and in precession to one of the poles (fig. 23). The pause between the spermatocyte divisions is short. Figures 24 and 25 show sister secondary spermatocyte metaphases. The division that follows is equational for the sex chromosome. It can be identified as the last chromosome to enter the reconstructing nucleus (fig. 26). The anaphase of the sister secondary spermatocyte division is readily identified by the absence of the characteristically large sex chromosome. From the meiotic divisions there are formed in the gynandromorphs, as in the normal individuals, spermatids of the X and no-X classes.

The development of the germ cells of gynandromorph A (fourth instar) had proceeded to the beginning of metamor-

phosis of the spermatids, while in gynandromorphs B and C (fifth instar) a large number of cysts contained spermatids in the final stages of metamorphosis. In the transformation of the spermatids into spermatozoa, mitochondria and Golgi bodies were observed to be apparently undergoing the same changes that were occurring in the normal specimens.

CHROMOSOMES OF FEMALE TISSUE

Two metaphase plates from the ovarian follicle cells of gynandromorph A were favorable for making a study of the chromosomes in the female tissue (figs. 19 and 20). Two sex chromosomes were present in each plate. They are indicated by both the number seventeen and the letter X in figures 19, 20, 27, and 28. In the chromosomal group represented by figure 20 the long end of one of the sex chromosomes was slightly twisted and folded. Pairing proved it to be one of the sex chromosomes (fig. 28). The XX constitution is the same as that in the normal female.

The exact number of autosomes could not be determined. Two autosomes in the first plate and three in the second were so situated that they could not be interpreted successfully. In order to study the chromosomes more carefully, they were drawn separately and compared with the chromosomes from the male tissue and from the normal material. In the first plate the two autosomes that were difficult to understand were given the numbers eighteen and nineteen. Eighteen lay between, in contact with, and below two autosomes (sixes) which were considered as a pair. Since some of the chromosomes, particularly the sex chromosomes, indicated an early separation on the metaphase plate, eighteen may have been a sister chromosome of one of the sixes. However, it was larger than either. Nineteen lay below and in contact with one of the corresponding twelves. It resembled eighteen so closely in size and shape that they might have been paired and placed in the series. One of the corresponding fourteens lay to the side of the plate, but compared rather favorably with a chromosome on the spindle.

In the second metaphase plate the three autosomes that were difficult to understand were given the numbers eighteen, nineteen, and twenty. Eighteen might have been considered as separating from ten, since they were closely approximated and similar in size and shape. Eleven and nineteen overlapped, and the exact boundaries were difficult to determine. Eleven may have been drawn slightly too large and nineteen too small; if so, eleven and nineteen might have been considered as separating halves. Twenty lay a considerable distance below the equatorial plate and out of focus with it. This position suggested that it did not belong to the normal chromosome group.

From the above it is seen that, if the autosomes numbered eighteen and nineteen in each plate were separate chromosomes and if twenty of the second plate were omitted, the total number of chromosomes in the female tissue would be thirty-six; whereas, if those numbered eighteen and nineteen be considered as separating chromosomes, the total number would be thirty-four. Among the normal specimens the former number (thirty-six) is characteristic of *A. oblongifolia*, and the latter number (thirty-four) is characteristic of *A. rotundifolia rotundifolia*. Since the chromosomes of the two species range from large to small gradually and are nearly alike in size and shape, it is impossible to determine by a comparison of the chromosomes the specific group to which the chromosomes of the female tissue belong. But the gynandromorphic specimen agrees with *A. rotundifolia rotundifolia*. From this agreement the female tissue might be expected to have thirty-four chromosomes.

Although a positive statement cannot be made as to the exact number of autosomes that were present in the female tissue, a careful study of the chromosomes of the two metaphase plates and the structural agreement of the gynandromorphic specimen with a species which is known to contain thirty-four chromosomes indicate that the female tissue contained the same number of autosomes as the male tissue. In this case the female tissue differed from the male tissue by

one sex chromosome alone. The additional autosomes that were observed in the female plates may be attributed to a precocious separation in the early anaphase or to an unequal chromosomal distribution at an earlier mitotic division. Both conditions are known to occur frequently.

Two metaphase plates in the female tissue of gynandromorph B showed two sex chromosomes to be present. The autosome number was determined to be diploid or nearly so.

Oogenesis was observed in the ovarian tubules of the gynandromorphs through the early part of the growth period. The process was normal.

EXPLANATION FOR THE GYNANDROMORPHS

Of the various hypotheses which have been advanced from time to time to account for gynandromorphs, four interpretations have been given a chromosomal basis:

1. Partial fertilization (Boveri, '88). According to this interpretation, the spermatozoon might be delayed in penetrating the egg until after the process of cleavage begins. The spermatozoon then uniting with one daughter nucleus would give rise to nuclei with the diploid number of chromosomes, while the other daughter nucleus would give rise to nuclei with the haploid number of chromosomes. The nuclei with the diploid number of chromosomes would give rise to female parts, whereas the nuclei with the haploid number of chromosomes would give rise to male parts.

2. Polyspermy, suggested by Morgan ('05) as a hypothesis alternative to partial fertilization. According to this interpretation, the egg might be penetrated by more than one spermatozoon, one of which, uniting with the egg nucleus, would form a nucleus with the diploid number of chromosomes, while the other spermatozoon would develop independently with the haploid number of chromosomes. In this hypothesis, also, the diploid nuclei would give rise to female parts, the haploid nuclei to male parts.

3. Theory of the binucleated egg, suggested by Doncaster ('14) from observations that were made on the eggs of

Abraxas. Some of the eggs were seen to contain two nuclei, each of which was about to unite with a spermatozoon. If one of the spermatozoa were of the male-producing class (i.e., of the no-X class) and the other of the female-producing class (i.e., of the X class), the resultant nuclei would give rise to male and female tissues. This hypothesis has been used as a possible explanation for a few gynandromorphs which cannot be explained by chromosomal elimination.

4. Chromosomal elimination, suggested by Morgan ('14) and since based upon an abundance of genetic evidence. According to this hypothesis, gynandromorphs start as normal female zygotes with the XX constitution, and during one of the cleavages an X-chromosome lags on the spindle and fails to enter either daughter nucleus. Thus, there would be produced nuclei with XX and XO constitutions which give rise to female and male parts.

The first two hypotheses have been used to account for gynandromorphs among the Hymenoptera where the female has the diploid, and the male, the haploid number of chromosomes. The genetic work of Whiting and Whiting ('27) on the hymenopterous parasite *Habrobracon* indicates that the first and third hypotheses may apply to gynandromorphs occurring among that group. The extensive work on *Drosophila* has demonstrated that the vast majority of cases are due to the elimination of one sex chromosome, while a few that do not fall under that explanation can be explained by assuming dispermy in the binucleated egg.

In case of gynandromorphic katydids A and B the presence of the diploid number of chromosomes in both the male and female tissues would preclude the possibility of them being produced by partial fertilization or polyspermy. Either the theory of dispermy in the binucleated egg or chromosomal elimination would account for the XX and XO constitutions of the female and male tissues. Since the abundance of conclusive genetic data from *Drosophila* proves the elimination of a sex chromosome from a female zygote to be the more usual cause for the production of such abnormal individuals, it is the

explanation which is preferred. It is assumed, then, that the gynandromorphic katydids began as normal female zygotes. During an abnormal embryonic cleavage one of the sex chromosomes was lost, giving rise to nuclei of XX and XO constitutions.

Whether the loss of a sex chromosome alone was responsible for throwing the sex balance to the male side cannot be stated conclusively, for if the additional autosomes indicated in the female metaphase plates belonged there normally they also were lost during an abnormal mitotic division. This loss would have to be considered a possible factor, because Bridges ('25) has shown that the autosomes also carry genes that are responsible in the production of sex. As previously pointed out, however, the extra autosomes probably did not belong to the normal female group, and the difference between the two tissues was represented by a sex chromosome alone.

SUMMARY

1. Two of the gynandromorphs were detected in the field during the nymphal period by color differences.
2. Each compound gonad had a common duct whose origin was in both testicular and ovarian tubules.
3. The first-described gynandromorph could not have functioned as either male or female in copulation because of abnormally developed reproductive organs. The second could have functioned as a normal female.
4. Self-fertilization was a morphological possibility in both forms.
5. Spermatogenesis, and oogenesis, as far as it had occurred, were normal.
6. The male tissue contained a single sex chromosome and the female tissue, two sex chromosomes. Both tissues were represented by the diploid (or nearly diploid) number of chromosomes.
7. Either the theory of dispermy in the binucleated egg or chromosomal elimination explains the origin of the gynandromorphs.

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PLATE 1

EXPLANATION OF FIGURES

The reproductive organs of normal and gynandromorphic katydids. Drawings free-hand; from alcoholic specimens, with the exception of figure 9.

- 1 Left side of ovipositor of normal female.
- 2 Same, ventral aspect with the valves separated.
- 3 and 4 Left and right sides of the ovipositor of gynandromorph A. Note the rudimentary ventral valve of the left side and the extent of the eighth sternite.
- 5 Same, ventral aspect.
- 6 and 7 Left and ventral aspects of the normal male.
- 8 Normal female, dorsal aspect.
- 9 Gynandromorph B, dorsal aspect.
- 10 Normal male, dorsal aspect.
- 11 The same from below, the gonads not represented.
- 12 and 13 Abnormal internal reproductive organs of gynandromorph A.

ABBREVIATIONS

<i>a</i> , abnormal dorsal valve	<i>sg.p</i> , subgenital plate
<i>ae</i> , aedeagus	<i>s.r</i> , seminal receptacle
<i>c</i> , cerus	<i>s.v</i> , seminal vesicle from the walls of which the secondary accessory glands develop
<i>cd</i> , common duct	
<i>d</i> , dorsal valve	
<i>i</i> , inner valve	<i>t</i> , testis
<i>o</i> , ovary	<i>v</i> , ventral valve
<i>od</i> , oviduct	<i>va</i> , vagina
<i>ot</i> , ovotestis	<i>v.d</i> , vas deferens
<i>p</i> , papilla of rudimentary ventral valve	<i>v.p</i> , vesicle which gives rise to the pri- mary accessory glands
<i>sa.p</i> , supra-anal plate	

The numbers indicate abdominal segments.

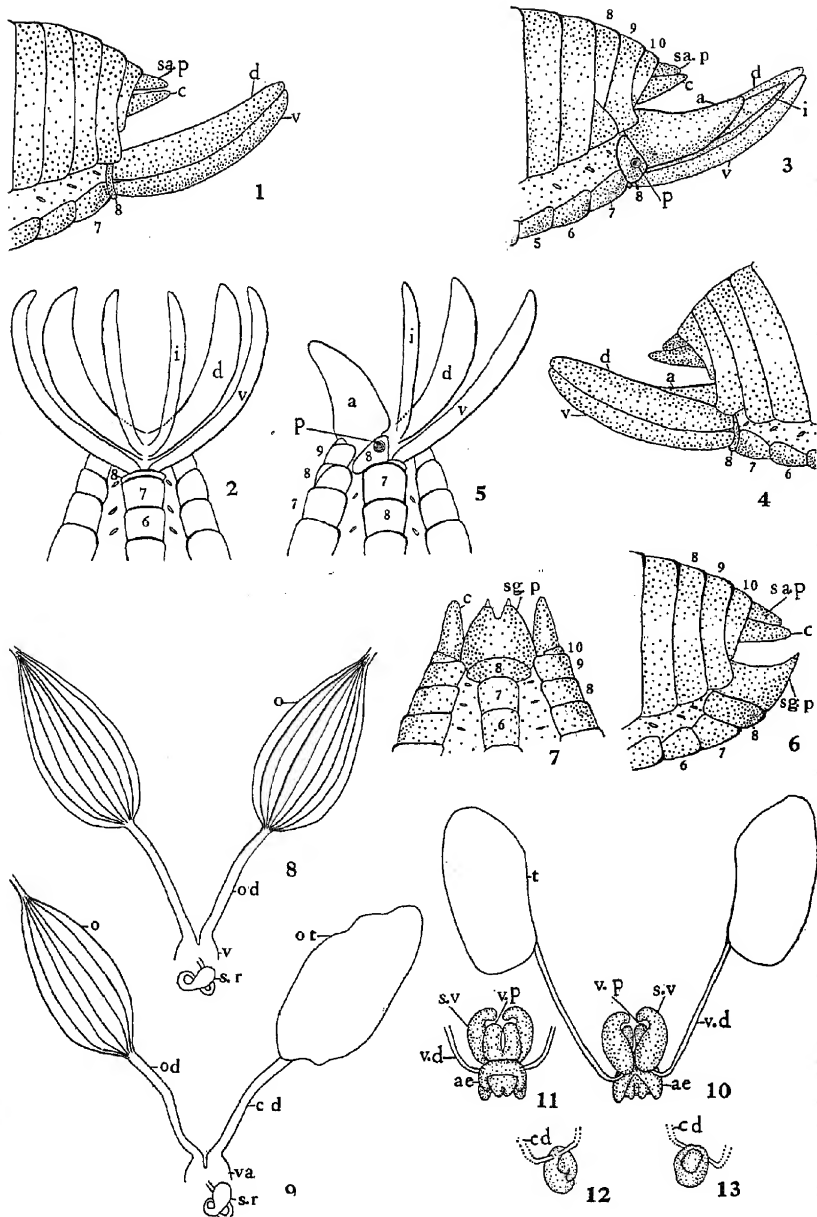


PLATE 2

EXPLANATION OF FIGURES

14 and 15 Cross-sections from the ovotestes of gynandromorph A. Note the common ducts receiving ducts from both male and female tissues. $\times 37$.

16 Epididymis of the normal male emerging from the testis. Longitudinal section. $\times 37$.

ABBREVIATIONS

C.D., common duct
E., epididymis

T.D., duct from testicular tubules
T.F., terminal filament

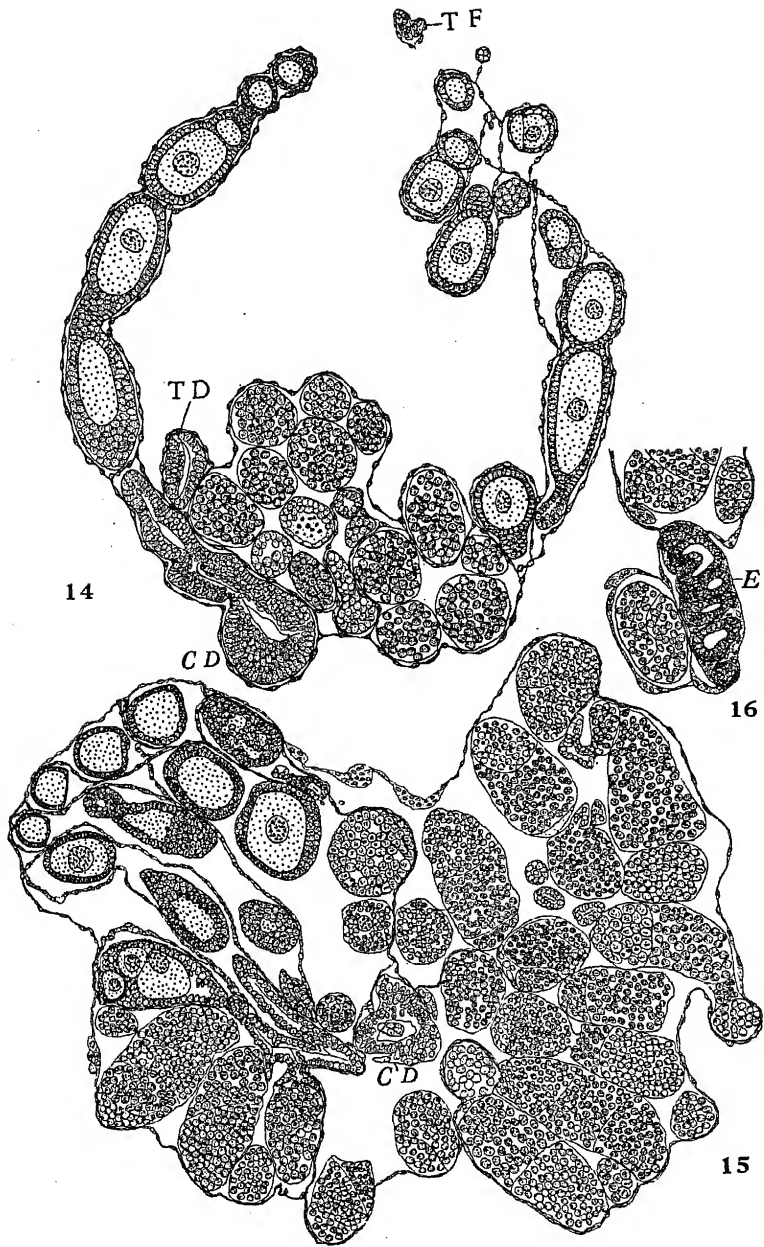


PLATE 3

EXPLANATION OF FIGURES

Chromosomes from the normal male, and from the male and female tissues of the gynandromorphic specimens. $\times 1920$.

17 Spermatogonial metaphase from gynandromorph A, thirty-three chromosomes.

18 Spermatogonial metaphase from normal individual of *A. rotundifolia rotundifolia*, thirty-three chromosomes.

19 and 20 Metaphases from ovarian follicular epithelium of gynandromorph A. Note two sex chromosomes.

21 Growth period from gynandromorph A.

22 Polar view of metaphase of the first spermatocyte division; gynandromorph A.

23 First spermatocyte division, gynandromorph A.

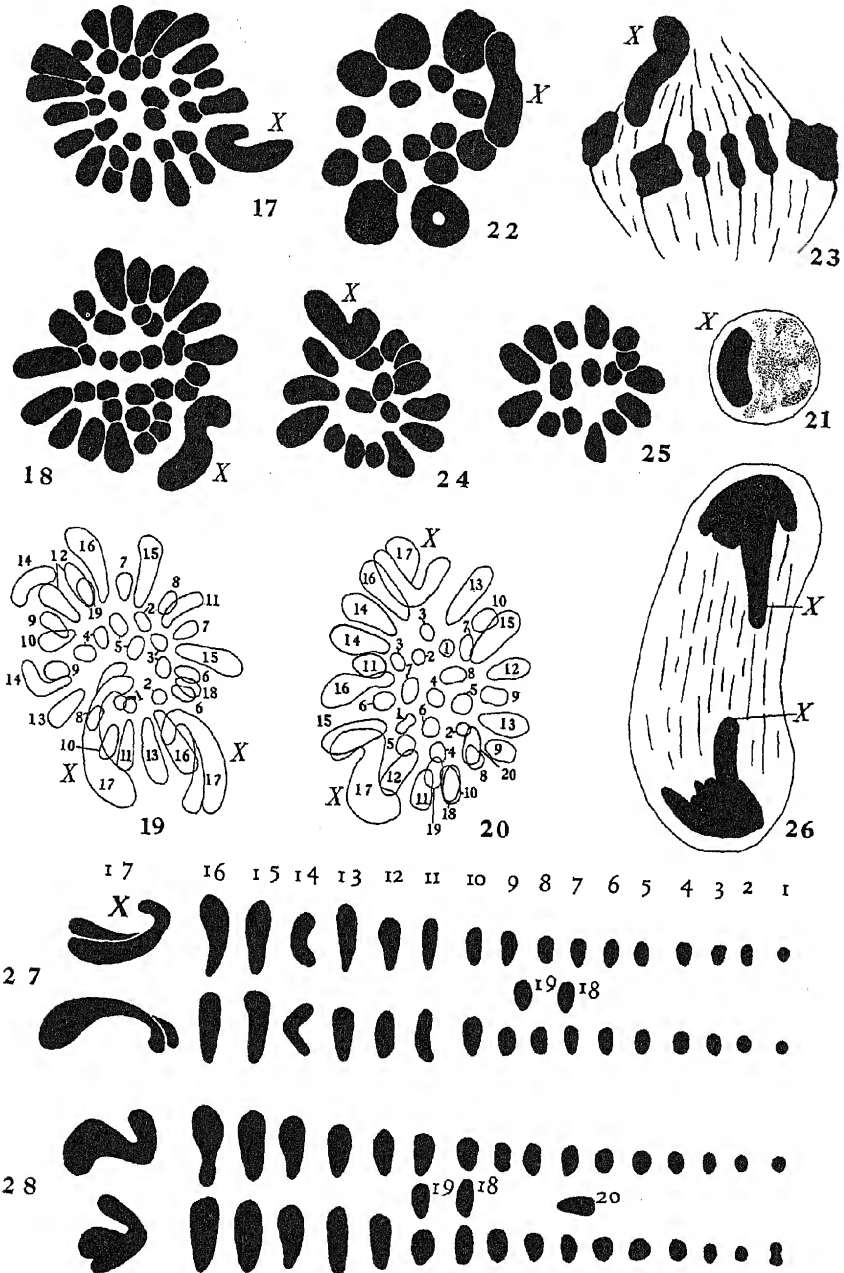
24 Polar view of metaphase of second spermatocyte division, X class; normal.

25 Polar view of metaphase of second spermatocyte division, no-X class; gynandromorph A.

26 Anaphase of the second spermatocyte division, X class, showing the sex chromosomes approaching the ends of the spindle; gynandromorph B.

27 and 28 The paired chromosomes of figures 19 and 20.

X, sex chromosome



THE SPERMATOGENESIS OF LEBISTES RETICULATUS

JEAN VAUPEL

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SIX PLATES (FIFTY-TWO FIGURES)

AUTHOR'S ABSTRACT

The germ cells of *Lebistes* are found in cysts; the younger cysts are toward the cortex. Mitochondria and Golgi apparatus are present. During maturation leptotene, bouquet, pachytene, and diakinesis figures are seen. The spermatocyte chromosomes number twenty-three; an X-Y pair is probably present, though the evidence is not conclusive. In spermatid formation the centriole divides to form a rodlet and an axial filament; the nucleus segregates into two materials, one of which is extruded; the remainder first contracts to a cup, comes in contact with the rodlet, then again forms a sphere. The mitochondria are arranged along the proximal part of the axial filament; the sphere flattens and elongates; the rodlet sinks into the head substance and is enfolded by it. The Golgi remnant is sloughed off with the residual cytoplasm, which disappears at the same time the Sertoli cells show an increase in size, suggesting their ingestion of the spermatid remnants. The mature sperm form spermatozeugmen, which are stored in the testicular canal; they are transferred to the female by aid of the modified anal fin.

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INTRODUCTION

Ballowitz and Retzius ('95) have described the mature sperm cells of a number of teleosts; a bibliography of earlier workers is given by Retzius; Turner ('19) has given a brief account of the spermatogenesis of the perch; Geiser's ('24)

study of the germ cells of *Gambusia* is devoted largely to the first portion of the development; Duesberg's ('18) account of the spermatogenesis of *Fundulus* has emphasized the behavior of the chondriosomes.

In order to confirm by cytological evidence the results in breeding experiments with *Lebistes reticulatus*, which are interpreted to indicate the presence of color factors in the Y-chromosome, Winge made a study of both male and female germ cells in this form; but, as he himself states, his intention was but "to point out the most striking features, and to account for the peculiar mode of inheritance."

With the exception of the papers mentioned above, the study of the development of the teleost sperm, particularly the later stages, has remained practically untouched. It is the purpose of this paper to give, as far as possible, a complete account of the spermatogenesis of this form.

DESCRIPTION OF THE FORM

Lebistes reticulatus is a poeciliid cyprinodont, native to fresh-water pools in the West Indies and tropical America. Among aquarists it is the most popular of the viviparous fishes; the bright color patterns of the male have earned it the name of 'rain-bow' or 'peacock' fish. They are easily bred in small aquaria; the females become mature in three to four months, and produce broods throughout the year at four to six weeks' intervals. In the natural state the fry number from five to as many as fifty at each bearing, according to the age and condition of the female; in aquaria, however, the broods usually number from three to ten.

The transfer of the sperm to the female is effected by the modified anal fin, or gonopod, but the exact mode is not clear. In the testicular canal of the male the mature sperm are found, gathered into balls, consisting of thousands of sperm arranged with their heads toward the outside of the mass, held together by the intertwining of the long tails. Winge states that in copulation the sperm balls or spermatozeugmen are "discharged like shot toward the genital duct of the

female"; this is clearly incorrect; my own observations, corroborated by those of several experienced aquarists, have assured me that the gonopod undoubtedly makes a contact with the genital opening of the female.

The small males hover almost incessantly about the larger females, seeming to display their bright colors, expanding their tails and vibrating their pectoral fins; at intervals the gonopod is extended until almost at right angles from the body, although the usual position is parallel to the long axis of the body. The male endeavors to approach the female; sometimes he darts forward and upward toward her; occasionally he backs toward her, endeavoring by a sudden turn to touch the gonopod to the genital opening.

A reasonable explanation of how the sperm are transferred during this contact seems to be as follows: the sperm balls are discharged and pass along the gonopod (fig. 1), being held there by the movement of the fin against the water; the membranous finger-like projection on the anterior margin of the fin, together with the strong tip, formed by rays II, III, and IV (fig. 1), is inserted into the genital opening of the female, which is enlarged by the momentary extension of the membranous projection (*mp*), allowing the spermatozeugmen to enter the body of the female.

They are evidently broken up in their movement up the oviduct; as noted by Winge, the individual sperm are seen lying in the folds of the ovary, with the tip of the head tightly pressed against the follicle cells separating them from the maturing ova. The spermatozoa evidently remain here for months, as one impregnation suffices for several broods.

MATERIAL AND METHODS

The individuals used in this study were from healthy stock in which newly hatched young had been appearing every few weeks for several years. Adult males ranged in length from 20 to 25 mm. Adult testes and whole young fish (from 6 to 18 mm. in length) were killed and sectioned, together with a few mature ovaries. The mature males were decapitated, and

in nearly all cases the body cavity was opened, and the testes were removed while the heart was still beating. The following methods were employed:

1. Allen's modification of Bouin's, with the addition of 1 per cent urea. This, followed by Heidenhain's, gave good preparations for the study of the chromatin. Sections were cut 5 or 10 μ thick; owing to the small size of the cells, the thinner sections were easier to study.

2. Fixation by osmic-acid vapor, followed by Heidenhain's, also gave some good smears and sections, especially the former.

3. Strong Flemming's, with the acetic acid reduced to three drops, followed by staining according to Benda's alizarin method, as revised by Duesberg and Meves, gave beautiful preparations for the study of chromatin, chondriosomes, centriole, and the axial filament; the same fixation and hardening, followed by Heidenhain's, gave useful preparations. Regaud's IV B, recommended by Cowdry for chondriosomes, gave unsatisfactory results, due to the swelling of the cells and their contents. Duesberg reported a similar effect in his *Fundulus* material.

4. The special methods suggested by Ballowitz and Retzius for the study of the teleost sperm, such as killing in formalin, followed by staining with gentian violet, or staining with gentian violet without fixation, and mounting in glycerin, gave unsatisfactory results with *Lebistes* material; with the exception of the osmic-vapor treatment as noted above, the only one of their methods that was helpful was the use of a concentrated solution of potassium acetate for maceration of fresh cells in some stages. A slower effect of the same sort, producing less distortion, was obtained by allowing the cells to remain for some hours in 0.75 per cent salt solution.

5. Preparations of fresh cells were very useful. These were made by teasing apart the cysts with fine needles in a drop of the body fluid, adding a cover-slip, and keeping the preparation from drying out by allowing normal salt solution to run in under the cover-glass from the sides. With this

method, the spermatids and mature sperm show movement for twenty to thirty minutes; the mature sperm have occasionally lived for over an hour. The fresh cells were also stained by dissolving the dyes in the salt solution; gentian violet, methyl green, dahlia, and Janus green B were employed in this manner.

ANATOMY OF THE TESTIS

The testes are two oval whitish bodies, 2 to 3 mm. in length in the mature male, lying in the dorsal part of the body cavity, below the swim bladder, just anterior to the cloaca. They are partly fused, though in the immature male the fusion is less complete. They are covered with an extremely delicate membrane, so transparent that the outlines of the cysts within are plainly seen. This membrane is easily ruptured, in which case the loss of some of the outer cysts follows. The ducts from the two sides unite to form a short vas deferens, which opens almost immediately into the urinogenital sinus.

Microscopical examination shows the structure of the testis to be like that described by Geiser in *Gambusia holbrooki*, another poeciliid. A cross-section of the mature testes in the most anterior portion shows only cysts, the younger stages lying in the cortical region; as one proceeds posteriorly, the branching ends of the main canal are seen in the central part, lying among the cysts; still farther to the posterior, the large lobulated main duct is seen, approaching the inner wall of the testis to fuse with the duct from the other side. The early spermatogonia are found in small rounded cysts, showing only a few cells in a section; sometimes several generations are noted in a cyst; the later stages are found in large cysts whose cross-sections show several hundreds of cells in almost exactly the same phase of development, so that only rarely can transition stages be found in the same cyst. A feature favorable to study is the fact that while any one testis may exhibit a preponderance of stages in a certain portion of spermatogenesis, still all stages are usually represented.

DESCRIPTION OF STAGES

Spermatogonia

No attempt was made to estimate the number of generations of these cells; several generations, when in one cyst, can be distinguished by their size, which varies according to age. The youngest are huge cells, showing in the rest stage a large clear nucleus, with usually one prominent chromatin nucleolus, from which delicate linin fibrils radiate (fig. 2). In Flemming-Benda preparations the cytoplasm is seen to contain numerous rounded chondriosomes, lying close to the nuclear wall. Many cells, as noted by Duesberg in Fundulus, have a majority of the mitochondria aggregated into a mound at one pole of the cell. The ground-work of this mound, which is too delicate to be seen in detail, shows a reddish tinge against the rest of the cytoplasm; I assume that it is the Golgi apparatus. As the cell prepares for division the chromatin spreads along the linin threads; the mitochondria diminish in size and lie against the nuclear wall (fig. 3). Next, the chromosomes assume a definite outline, lying just below the nuclear membrane for a short time before their arrangement on the spindle. The mitochondria have decreased in number and in staining capacity; their position has shifted away from the nucleus (fig. 4). In division the mitochondria become concentrated into a few deeply staining globules, lying toward the outer part of the cytoplasm (figs. 5, 7). In the various spermatogonial divisions, as the chromosomes move apart, the chondriosomal masses seem to be pushed away from the poles of the spindle and to lie between the clumped masses of chromosomes (fig. 8); in the daughter cells this arrangement persists for a time (fig. 9). The spermatogonial chromosome number is approximately forty-six, as established by Winge (fig. 6). The later generations of spermatogonia show a progressive decrease in size, culminating in the final spermatogonia; this can be seen by contrasting figures 2 to 6 with figures 7 to 10. In the rest stages the mitochondria of the later generations are not invariably

gathered at one pole, but are scattered in several irregularly shaped patches, closely applied to the nuclear wall (fig. 10).

Growth period

The young spermatocytes (fig. 11) can be distinguished from the later generations of resting spermatogonia only by the larger size of the cyst in which they are located; they are of about the same size; both show a prominent chromatin nucleolus and perhaps some smaller chromatin clumps and scattered patches of chondriosomal substance. The increasing size of the spermatocyte soon serves to identify it more clearly (fig. 12). The nucleus becomes cloudy as the delicate leptotene threads begin to appear. They seem to be spun out of clumps of chromatin which lie against the nuclear wall; I cannot say whether these clumps have arisen by the division of the nucleolus or whether they have appeared in place.

The growing nucleus now appears inflated and contains taut threads, extending inward from the clumps outlining the nuclear wall; the beginning of polarization can be noted (fig. 13). It is difficult to distinguish the mitochondria at this stage; the enlarged nuclei fairly seem to touch; only occasionally can the cell walls be distinguished.

The completely formed faintly granular leptotene threads soon come to lie in a more orderly arrangement, with their ends directed toward the pole, where, in favorable cells, the Golgi apparatus, a cap-shaped reddish mass of denser consistency than the remainder of the cytoplasm, can be distinguished (fig. 14). Next, the mitochondria gather at the pole in a conspicuous mound, hiding the Golgi apparatus (fig. 15). The leptotene threads draw closely together; individual threads can no longer be distinguished, except near the pole. Synapsis must occur at this stage, as the thicker pachytene threads next appear, but the cells are too small and the threads too delicate and faint at this point to give much evidence of union, even with the most careful examination. As noted by Geiser for *Gambusia*, there is no synezeisis.

Following this, the nuclei seem to be distended with the long polarized pachytene loops (fig. 16). The cytoplasmic structures are somewhat obscured; it is certain, however, that the aggregates of mitochondria are broken up, since the conspicuous polar caps are no longer visible. The loops next appear to become shorter and thicker (fig. 17); at no time is there any evidence of a diplotene stage; there is no clear evidence of twisted threads, unless the uneven thickness of some of the loops be taken to suggest a strepsitene figure.

The entire mass of threads next seems to contract still further, the polarized arrangement still persisting; the threads are seen but faintly (fig. 18). The chondriosomal material is seen to be distributed in several scattered condensed granules, lying in the cytoplasm near the nucleus. The contraction of the chromatin mass continues, giving the effect of an increase of cytoplasm; traces of polarization are no longer to be seen; the nucleus appears as a pale and almost homogeneous ball (fig. 19). Owing to the contraction of the nuclear material at this point, the Golgi apparatus is readily seen; in it one or two centrosomes are embedded. The masses of chondriosomal substance are no longer in the midst of the cytoplasm; they are at the extreme periphery of the cell, tightly compressed into the angles formed by the pressing together of the cells, as if to make way for the formation of the spindle. Occasionally one notes scattered small dark granules clinging to the surface of the nucleus, which may also be mitochondria.

This diffuse period is succeeded by one of short duration in which the homogeneous pale appearance of the nucleus is lost; the chromatin mass, which again takes the stain, shows clear spaces, the interstices of a coarse tangled network, which is connected to the nuclear wall by delicate threads (fig. 20). That this is an early diakinesis stage is seen clearly in the following stage, in which the beginning of condensation of the chromosomes is visible (fig. 21). Diakinesis proper follows rapidly; only in large clear cells can some of the condensing chromosomes be studied, and then only in the center

of the highest and lowest levels of the nucleus (fig. 22). In late diakinesis all the chromosomes have condensed; their bivalent nature is indicated by a constriction across the middle, which gives them somewhat of a dumb-bell appearance; all now lie near the nuclear wall (fig. 23).

Spermatocyte divisions

The nuclear wall now disappears; the spindle is formed; the now rounded chromosomes are arranged on the spindle; division proceeds. The first spermatocyte metaphase plate shows twenty-three rounded chromosomes of varying sizes (fig. 24). Winge states that his *Lebistes* material did not reveal any differences which might serve to distinguish the sex chromosomes. In my material, the chromosomes sometimes enter the telophase in a body; sometimes spindles are seen with several chromosomes already at the pole, while the remainder are still in the metaphase; in many cells the behavior of one pair, whose members pass to the poles ahead of the others (fig. 25, a, b), suggests the presence of an X-Y pair, though the evidence is not conclusive.

After the first division, contrary to Winge's findings, an immediate reorganization of nuclear and cell walls to form two complete daughter cells follows. The individual chromosomes are lost from view; a lightly staining diffuse mass fills the nucleus. The chromosomes quickly reappear, and, as in the case of the spermatogonial and first spermatocyte divisions, the condensing chromosomes are seen lying immediately below the nuclear membrane, preceding their arrangement on the spindle (fig. 26). Spindle formation follows (fig. 27, a, b); the divided chromosomes pass to the poles in two irregular clumped masses (fig. 28, a, b). Rarely is a plate found in which a count can be made, owing to the extremely small size of the chromosomes and the tendency to clump; however, in favorable cells there are undoubtedly twenty-three rounded chromosomes visible, the smaller being on the inside of the group. In no instance could a count be made of the numbers in the telophase groups.

The movements of the mitochondria at the time of the first and second spermatocyte divisions seem to be similar to those observed during the spermatogonial divisions, namely, in the metaphases they lie in several masses outside the spindle (fig. 29); as the chromosomes move to the poles, these masses remain inertly behind, and some are distributed to each of the daughter cells (fig. 30).

Spermatid

After the second spermatocyte division, both nuclear and cell walls are immediately reorganized; the chromatin mass resulting from the clumping of the telophase chromosomes spreads smoothly through the nucleus, giving it a firm rounded homogeneous appearance. The cytoplasm is not clear; in Benda preparations there is a bluish cloudy precipitate, the scattered small mitochondria; some are adhering to the surface of the nucleus (fig. 31). The centriole appears as a deeply staining body, embedded in the Golgi apparatus, which is lying in the cytoplasm near the nucleus. While the mitochondria are still loosely scattered, the centriole sends out on one side a short blunt prolongation, which will form the tip of the axial filament, and on the other, the delicate axial filament. The clouded condition of the cytoplasm makes it impossible to state what the order of development is. As can be seen in the following stage, the filament grows through the cytoplasm, past the nucleus, and finally penetrates the cell wall on the side opposite the centriole. Owing to the crowding of the cells and the extreme delicacy of the filament at its first appearance, I have not been able to observe the pushing through the cell membrane; fresh cells at this stage show only the eversion of the cytoplasm (fig. 32); in fact, the filament is rarely visible in fresh cells at any stage.

The peripheral cytoplasm now becomes free of mitochondria, which in fixed cells appear as dark granules scattered over the nuclear wall and closely adherent to it, giving it a ragged outline (figs. 33, 34, a, b). The axial filament is clearly seen; it has increased either in thickness or staining

capacity (fig. 33). The rodlet at its anterior end is now pushed against the cell wall, perhaps because of the resistance encountered by the lengthening tail in forcing its way outward between the closely packed spermatids, so that in fresh cells, or in smears, freed from the pressure in the crowded cyst, the anterior end is bluntly pointed (fig. 34, a, b). The Golgi apparatus is carried forward with the centriole and rodlet, away from its earlier position against the nuclear wall (compare fig. 31 with 33); as before, it appears as a delicate light-reddish body.

The tip of the filament now moves away from the most anterior portion of the cell and comes to lie curved about the nucleus (fig. 35); its position is seen most clearly, perhaps, in cells whose membrane has been dissolved in a solution of potassium acetate. The mitochondria are seen to be moving toward that portion of the axial filament which is touching the nucleus (figs. 36, b; 36, c). The curving of the axial filament is perhaps partly mechanical; after the rodlet is pressed forward against the cell wall, if the pressure continues, the rodlet must then be bent to one side.

At about the time of the curving of the axial filament, sometimes prior to this, the nucleus shows a decided change from its former firm homogeneous condition; it appears to expand and to show clear spaces (fig. 35). The nuclear membrane then seems to burst open at one point, which may be on the side away from the axial filament (fig. 36, a) or on the side toward it (fig. 37). While the opening is still visible, the rodlet and centriole, carrying with them the Golgi apparatus, come in contact with the intact portion of the nuclear wall opposite the ruptured area (fig. 37). Some clear nuclear material which formerly occupied the colorless spaces must be extruded; the cytoplasm sometimes appears slightly cloudy, but soon resumes its former homogeneous appearance. The rupture of the nucleus may, of course, be the result of fixation. If so, the nucleus is unusually susceptible to the action of reagents at this particular stage, because the ruptured condition is not seen in other stages.

The deeply staining chromatin portion of the nucleus contracts still more to form a cup-shaped mass (fig. 38, a, b, c), with the rodlet applied to the bottom of the cup. At the same time the chromatin mass contracts, the mitochondria appear to have completed their movement from the nuclear surface to the axial filament, and lie clumped about its proximal portion, covering the Golgi apparatus (fig. 38, a, b, c). At first the rodlet is hidden (fig. 38, a), but the granules seem to pass backward rapidly, the rodlet again becoming visible (fig. 38, d, e).

The contracted chromatin cup now rounds out to form a sphere again (fig. 39), the chromatin at first being more concentrated near the rodlet, and later spread uniformly through the reformed nucleus (fig. 40, a). The mitochondria have slipped backward to their final position, arranged roughly in two rows about the upper portion of the axial filament. When the mitochondria pass back, the Golgi apparatus loses its connection with the centriole and lies free in the cytoplasm (fig. 40, a, b), and will eventually be cast off with the residual cytoplasm; it may now be termed the Golgi remnant. As before noted, in Flemming-Benda preparations, it stains a faint red; with Flemming-Heidenhain, it is dark; in live cells it appears as a rounded yellowish body, different in texture, deeper in color, and larger in size than the small slightly yellow crystalline mitochondria.

The spherical spermatid head now flattens to a disk shape (fig. 41, a, b, c). This change can be ascribed to no other cause than a pressure from the rodlet upon the nucleus; that this is the likely explanation is borne out by succeeding stages, in which the various changes of shape in the nucleus may all be explained by the pressing of the rodlet into the developing sperm head. After the disk stage, the continued pressure from the rodlet causes a longitudinal furrow to be formed, extending from the base of the head forward (fig. 42, a); in profile, that part of the head beyond the rodlet is bent forward (fig. 42, b); it is possible that the pressure against the cell wall helps to cause this flexing; the outline of the

head is now oval, rather than circular. In a cross-section, the rodlet is seen to be closely applied to the head (fig. 42, c).

The elongation of the head proceeds (fig. 43, a); the continued pressure from the rodlet has formed a deeper groove, causing the head material to bulge out into two lobes at the base (fig. 3, b, c); the keel-like outpushing at the tip is more pronounced (fig. 43, d, e). In the succeeding stage, the outline of the head has narrowed at the tip, suggesting an arrow-head (fig. 44, a); this must have been the stage termed by Winge the 'split arrow-head,' and which he describes as the fully developed sperm (fig. 44, b). The lobes at the base are folding in to inclose the rodlet completely in the head substance. (In Bouin-Heidenhain slides, the rodlet is colorless (fig. 44, b); in Flemming-Benda it shows dark (fig. 44, a).) This is also seen in cross-sections (fig. 44, c); in profile the groove is seen to be closing (fig. 44, d). Occasionally, both in live and fixed cells, the rodlet is seen to lie in a spoon-shaped depression produced by the deep sinking in of the inner wall of the sperm head, so that the inner and outer walls appear to be in contact (fig. 44, e).

The head now narrows and elongates; at this point of its development it is at its greatest length; the fissure in which the rodlet is lying extends to the tip of the head (fig. 45, a); the deeply staining rodlet is visible through the paler nuclear substance at the base of the head (fig. 45, b); in cross-sections it is seen to be sunk into and surrounded by head substance; a line of fusion is visible where the lobes at the base of the head are meeting to inclose the rodlet (fig. 45, c).

The process of enfolding continues in the next stage (fig. 46, a, b, c); the head, while still narrow, is not quite so long; there seems to have been a contraction of the material. At this point the head everts the cell membrane; it will soon break through (fig. 46, d, e). When the tip of the head pierces the cell wall, the cytoplasm slips back over the head. A trace of the former coiled position within the cell membrane remains in the angle in which the descending cytoplasm holds the head and the upper part of the tail (fig. 46, f). The cyto-

plasm must pass rapidly over the portion of the axial filament below the mitochondria; in live cells it is often seen caught along the mitochondria (fig. 46, g), but never along the lower portion of the tail; when the sperm are freed, the vibratile movements of the tail, too rapid to be followed by the eye, must serve to fling the cytoplasm off almost at once.

Mature sperm

The fully developed sperm in the spermatozuogen, whether seen in the folds of the ovary or in fresh preparations (fig. 47, a, b, c), has a head slightly smaller than when still in the cell membrane, quite pointed when one is looking down upon the faint trace of the enfolding of the rodlet (fig. 47, a); when viewed in profile, the tip is somewhat blunt (fig. 47, b). That the rodlet remains a distinct structure inclosed in head material is shown when mature sperm are allowed to remain in 0.75 per cent salt solution for some hours; the head is dissolved, the tail and mitochondria remain, with the rodlet projecting beyond the upper mitochondria (fig. 48). The mitochondria are usually arranged in two rows; no attempt has been made to count them; with the most careful focusing, the outlines are difficult to distinguish. The upper granules are pressed closely against the base of the head, covering the centriole; the lower ones are often smaller, and the last one or two may be in a single row (figs. 46, g, 47, b); following them is the long slender slightly tapering tail, which shows no indication of an end piece. Occasional sperm are seen with the mitochondria still clumped about the centriole as they were in younger stages (fig. 47, c). When the sperm are examined immediately upon the removal of the testes from the body, a delicate clear vesicle is seen at the neck; this disappears in a few minutes; I am unable to account for its appearance, except by the supposition that some of the cytoplasm, if there be any still present, is lost by plasmolysis (fig. 49, a, b).

Movements of the cells within the cysts

From the time at which the nucleus assumes the flat-disk shape there is a progressive decrease in the staining capacity of the nucleus, so that the later elongated stages, while absorbing sufficient stain to be studied quite easily, are pale as contrasted with the intensely staining earlier rounded stages. From the beginning of tail growth there is an increasing orientation of the cells with respect to the cyst wall. The outgrowth of the tail seems to be a factor in producing this orientation; it grows toward the center of the cyst, where there is less crowding, and consequently easier penetration between the cell bodies. Numbers of tails follow the same paths and are twisted about one another; several of the dark-staining bundles can be seen arranged radially in cross-sections of spermatid cysts; in fresh preparations, when such cysts are teased apart, the bundles remain a unit, held together by the entangled tails (fig. 50).

At the stage when the outpushing of the cell membrane by the pointed sperm head begins, the sperm heads are lying with their anterior ends directed toward the wall; the sperm then move forward and touch the wall, forming an almost uniformly distributed dark-staining ring (fig. 51). The cytoplasm, containing the Golgi remnant, passes backward and slips down over the tail; this process continues until protoplasmic balls of varying sizes, free of the tail, are seen passing to the central space. They quickly disintegrate; the cells of the cyst wall, which were thin and flattened, become strikingly enlarged (fig. 52); at the same time the sperm lose their uniform distribution, and in cross-section are seen to be arranged in a series of curves, the center of each curve being one of the Sertoli cells, as Winge noted in his material. The pronounced increase in the size of the Sertoli cells, accompanied by the disintegration and disappearance of the protoplasmic balls, suggests strongly the ingestion of the latter by the former.

The mature sperm now withdraw from the wall, the tails becoming entwined in the process; a connection with the main canal is established; the spermatozeugmen pass out into the central canal, which in some individuals is distended with sperm balls; it evidently serves as a place of storage until they are ejected during copulation. When the testes are teased apart and the spermatozeugmen are allowed to float free, they present a striking picture; the jerking movements of the long intertwined tails cause the whole Medusa-like mass to quiver and vibrate incessantly.

DISCUSSION

The above account agrees, in the main, with those of other investigators in this field, so far as the stages preceding the transformation of the spermatid are concerned. The general description of the testis and the formation of the definitive spermatogonia are similar to those reported by Geiser for *Gambusia*, and Duesberg for *Fundulus*. The maturation stages are involved and puzzling; yet I cannot agree with Winge's statement that "a characteristic feature is the absolute lack of stages which may be called synapsis and diakinesis. At most a faint deposit of chromatin may be observed as a compensation for the synizesis." The extreme fineness of the threads in the leptotene stages contrasts definitely with the thicker pachytene; though it is impossible to count the threads, the decreased number indicates that synapsis must have occurred. Then, too, the behavior of the mitochondria is also an aid in tracing the sequence of stages; while Winge does not state what fixatives and stains were used in preparing his material, it is safe to say that the mitochondria were destroyed, since he makes no reference to them; nor does he refer to fresh material, in which the mitochondria are very evident.

The most complete account of these early maturation stages yet given for teleosts is that of Geiser for *Gambusia*, also a poeciliid; the general order of the stages appears to be the same in *Lebistes*. There seems to be no reference in the scant

literature on teleost spermatogenesis to a contraction stage following the pachytene figure, preceding diakinesis; in my material this is most clearly demonstrated, occurring usually in the same cyst with diakinesis figures, and sometimes with spindles. Diakinesis must proceed more quickly in *Lebistes* than in *Gambusia*; no cells were noted with all chromosomes of an open-ring type, such as Geiser figures.

The movements of the chondriosomes agree in general with Duesberg's description of them in the germ cells of *Fundulus*, particularly as to the formation of the polar cap in rest stages, their behavior during divisions, and in the early spermatid. However, one could hardly be justified in saying that there is an exactly equal division of chondriosomal material at any of the divisions observed.

Nothing similar to the stages from the beginning of the growth of the axial filament has been described. In the *Fundulus* spermatid Duesberg reports that the chromatin condenses on the periphery of the nucleus, everywhere except the point from which the axial filament grows out; the *Lebistes* material shows just the contrary, the condensed chromatin material being localized where the centriole and rodlet touch the nucleus. In his late stages the basichromatin remains in a crust in the elongating sperm head; in *Lebistes*, if there is a separation of basi- and oxychromatin, it is the pale oxychromatin which is discharged from the nucleus and the basichromatin alone which forms the mature sperm head. The process of infolding about the axial filament seems unique; yet both fixed and fresh material seem to offer incontrovertible evidence on this point.

The anterior outgrowth from the centriole, termed the 'rodlet' (Miescher's Stäbchen), may perhaps be considered as representing the material of another centriole; in fresh material and in smears it sometimes shows as a little rounded knob instead of the usual blunt point (figs. 41, a, 42, d).

The figures given by previous workers for the mature teleost sperm often resemble stages in the transformation of the *Lebistes* spermatid. Nearly all show a rounded to oval

head, with one to several mitochondrial granules clumped at the base, and a long slender tail. There is no evidence of an acrosome and seldom a tail piece. Ballowitz's figures of the *Zoarces viviparus* sperm show a bowl-shaped nucleus, much like the flat-disk stage in *Lebistes*. His *Clupea* figure is spade-shaped, with a clear central canal extending halfway to the anterior end of the head; he shows a similar figure for the sperm of *Salmo salar*; both resemble Bouin-Heidenhain preparations of the stage in which the nucleus begins to elongate.

The sperm with numbers of granules in the neck (*Clupea*, *Idus melanotus*, *Esox*, *Lota vulgaris*) bear a striking resemblance to *Lebistes* stages in which the mitochondria are clumped near the basal body, preparatory to passing back along the axial filament.

Practically all sperms show a dark-staining basal body at the anterior end of the axial filament; sometimes at the base of the head (sturgeon); sometimes applied to the side of the sperm head (*Globius niger*), or seemingly inserted in the head (*Clupea*). Often it is hidden in the mass of mitochondria (*Cyprinus carassius*, *Idus melanotus*).

It is suggested that the conditions found in the *Lebistes* sperm may represent an evolution from a more primitive type exhibited in oviparous teleosts. With the development of viviparity, and the consequent long duration of the separate existence of the male gamete, the infolding of the sperm head perhaps serves to effect a stronger union between the parts of the sperm. A study of the sperms of other poeciliids would be of interest.

Bowen gives a résumé of the observations relative to the ingestion of the spermatid remnant and its inclusions by the Sertoli cells; he concludes that "we have here to deal with a phenomenon which is common to animals in general and is indicative again of the fundamental similarities in the differentiation of most animal sperms." No mention is made of an increase in size of the epithelial cells, accompanying the absorption of this material.

SUMMARY

1. A study of the development of the sperm of *Lebistes reticulatus* was made; as in other teleosts, the germ cells are found in cysts, the earlier stages being toward the periphery of the testis.

2. Maturation stages are described; leptotene, bouquet, pachytene, and diakinesis figures are found.

3. The haploid number of chromosomes is twenty-three.

4. There is some evidence for the presence of an X-Y pair.

5. The single centriole gives rise to an anterior rodlet; the centriole also acts as a blepharoplast.

6. The mitochondria are arranged in the form of a polar cap in prophases; a rough distribution to the daughter cells is indicated; in the sperm they lie closely applied along the proximal portion of the axial filament.

7. The Golgi apparatus is seen at intervals during spermatogenesis; the Golgi remnant is sloughed off in the residual cytoplasm.

8. There is a segregation of the early spermatid nucleus into two substances, followed by an extrusion of clear material into the cytoplasm.

9. The mature sperm head is produced by a process of flattening and infolding of nuclear material about the rodlet; it is suggested that it has evolved from the usual primitive type of teleost sperm.

10. There appears to be an absorption of the spermatid remnant by the cells of the cyst wall, followed by an increase in the size of these cells.

My thanks are due to Dr. H. L. Wieman for his constant advice and helpful suggestions throughout the course of this study.

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PLATES

EXPLANATION OF PLATES

All figures were drawn with the aid of a camera lucida at table level; with fixed cells, a 2-mm. objective and an 18 ocular were employed; with fresh cells, a 2-mm. objective and a 12 ocular. Figures 6, 22, 23, 24, 25, a, 25, b, 26, 27, a, 27, b, 28, a, 28, b, are from material fixed in Bouin's and stained with Heidenhain's; figure 44, b, was fixed in Flemming's and stained with Heidenhain's. All remaining cells from fixed material were fixed in Flemming's and stained according to Benda's method. When fresh material was used, it is noted in the description accompanying the figure.

ABBREVIATIONS

<i>c</i> , centriole	<i>n</i> , nucleolus
<i>ch</i> , chromosome	<i>mp</i> , membranous projection
<i>f</i> , axial filament	<i>r</i> , rodlet
<i>g</i> , Golgi apparatus or Golgi remnant	<i>v</i> , vesicle
<i>m</i> , mitochondria	

PLATE 1

EXPLANATION OF FIGURES

- 1 Adult gonopod. *II*, *III*, *IV*, rays *II*, *III*, *IV*.
- 2 Primary spermatogonium, rest stage.
- 3 Primary spermatogonium, beginning formation of chromosomes.
- 4 Primary spermatogonium, condensed chromosomes.
- 5 Primary spermatogonium, metaphase. (Cut cell.)
- 6 Primary spermatogonium, metaphase; forty-six chromosomes.

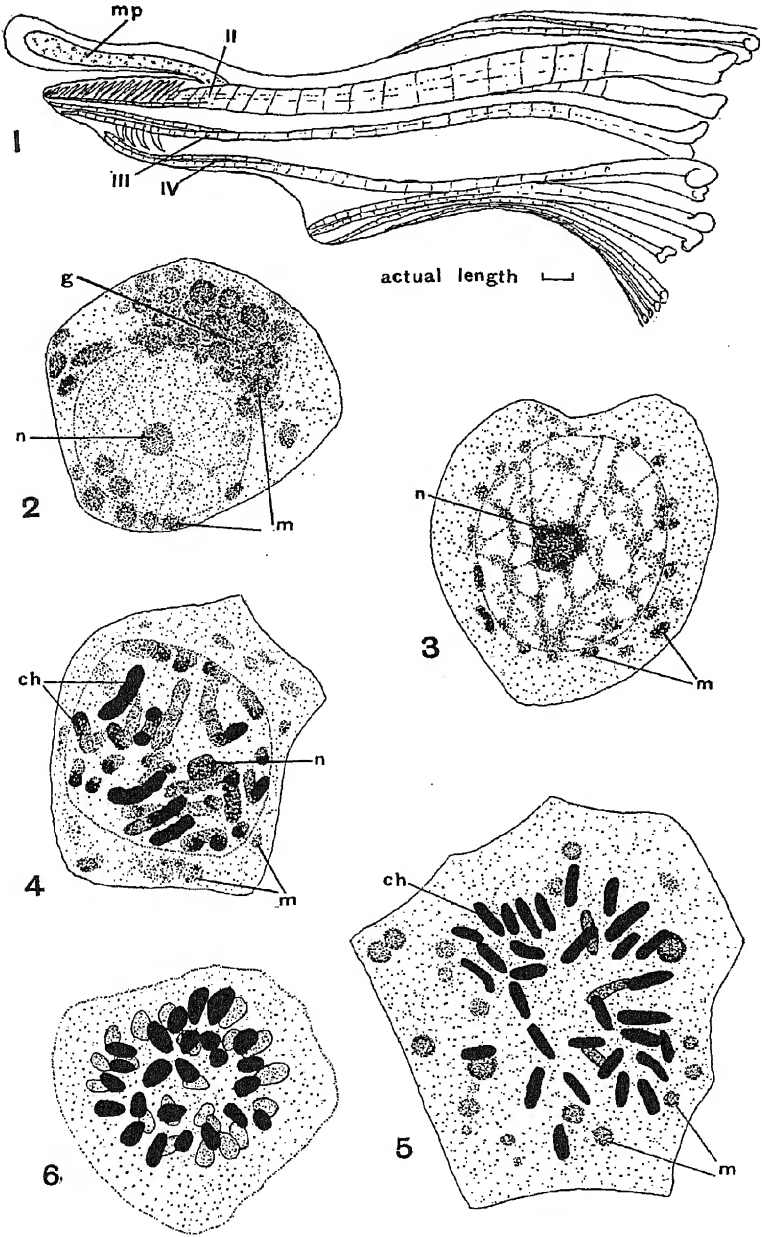


PLATE 2

EXPLANATION OF FIGURES

- 7 Spermatogonial anaphase, showing position of mitochondrial masses.
- 8 Spermatogonial telophase, with mitochondrial masses.
- 9 Daughter cells resulting from spermatogonial division, with mitochondrial masses.
- 10 Final spermatogonium, rest stage.
- 11 Young spermatocyte, rest stage.
- 12 Spermatocyte, beginning of formation of leptotene threads.
- 13 Spermatocyte, beginning of polarization of threads.
- 14 Spermatocyte, with polarized threads.
- 15 Spermatocyte, synapsis, with mitochondrial polar cap.
- 16 Spermatocyte, pachytene.
- 17 Spermatocyte, contracting loops.
- 18 Spermatocyte, chromatin fading.
- 19 Spermatocyte, no chromatin visible; Golgi apparatus prominent; mitochondria at periphery.
- 20 Reappearance of chromatin; very early diakinesis.

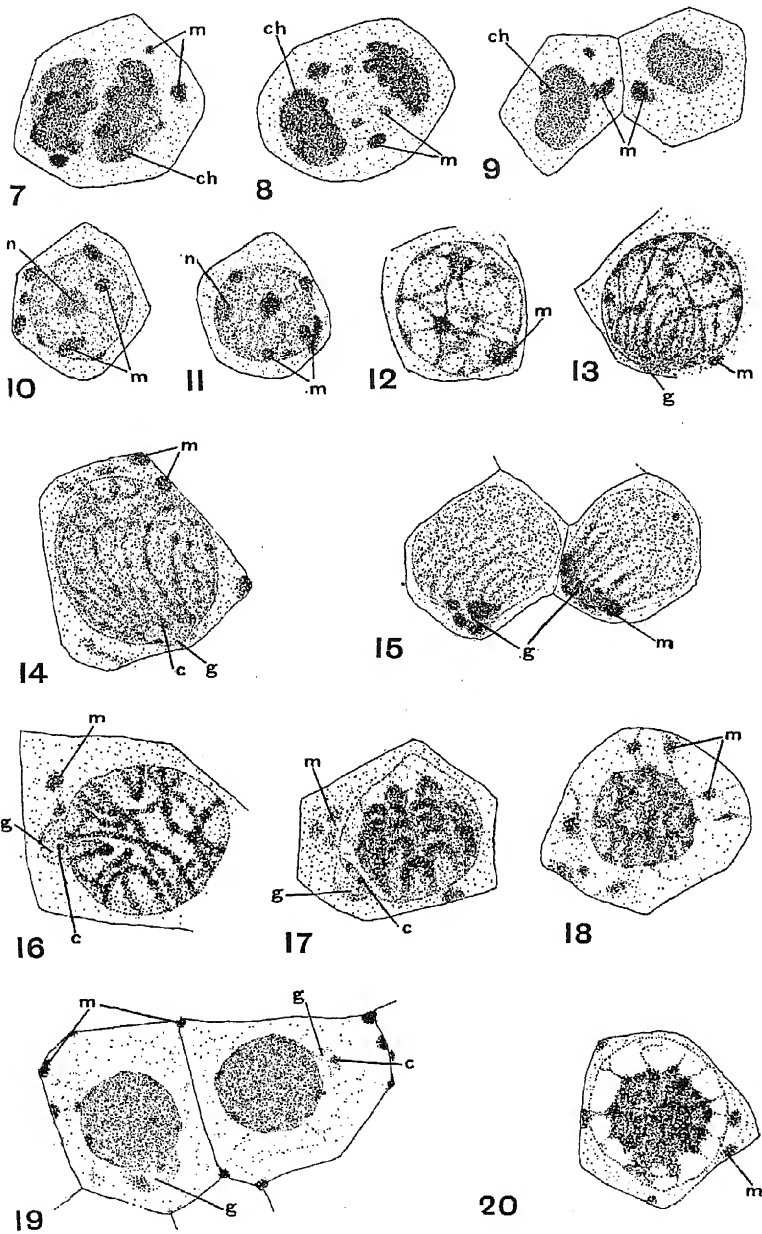


PLATE 3

EXPLANATION OF FIGURES

- 21 Spermatocyte, showing condensing chromosomes.
- 22 Spermatocyte, diakinesis.
- 23 Spermatocyte, just prior to spindle formation; twenty-three chromosomes.
- 24 First spermatocyte metaphase plate.
- 25, a, b First spermatocyte spindle, sex chromosomes in advance.
- 26 Second spermatocyte, just before formation of spindle; twenty-three chromosomes.
- 27, a Second spermatocyte metaphase plate.
- 27, b Second spermatocyte spindle, profile.
- 28, a Second spermatocyte telophase, early.
- 28, b Same, later.
- 29 First spermatocyte spindle, showing mitochondrial masses.
- 30 Second spermatocyte spindle, with mitochondrial masses.
- 31 Young spermatid.
- 32 Spermatid (fresh material) at beginning of tail growth.
- 33 Spermatid; Golgi apparatus free of nucleus; rodlet at anterior of cell.



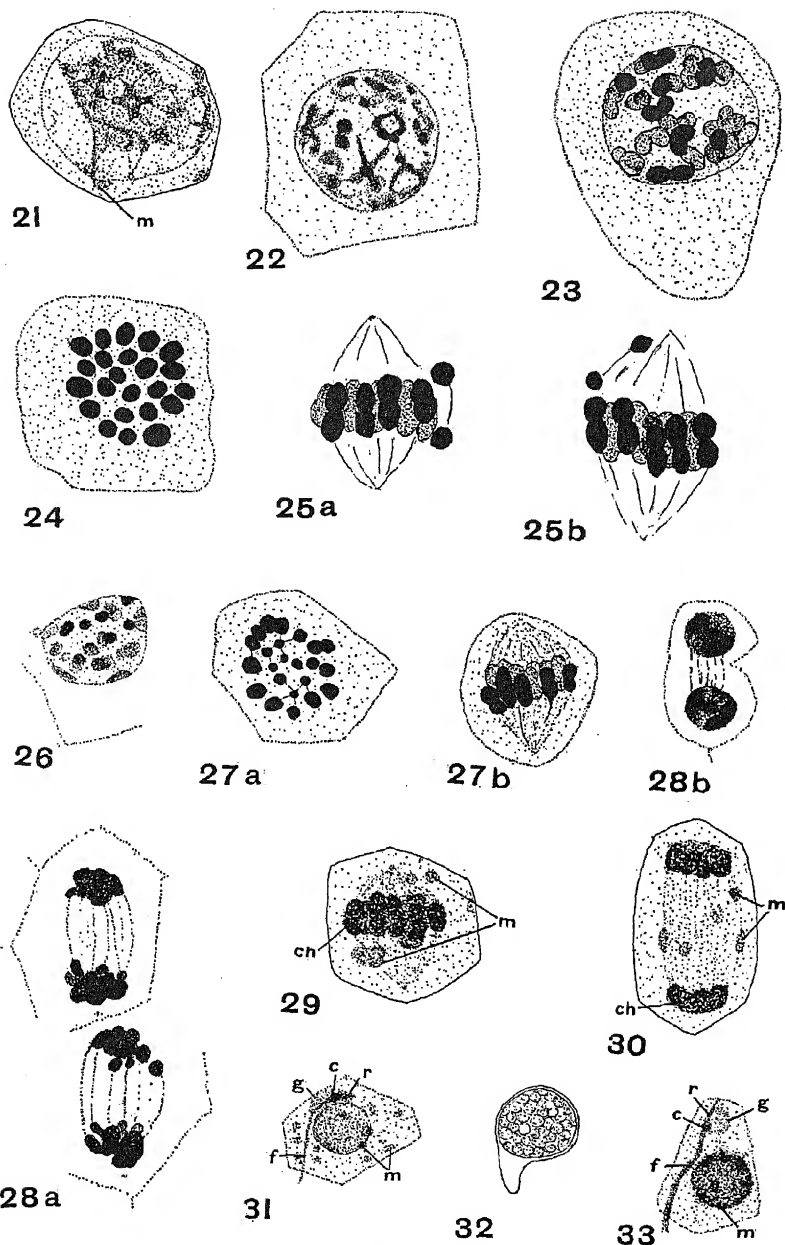


PLATE 4

EXPLANATION OF FIGURES

- 34, a, b Spermatids (fresh material); mitochondria adhering to nucleus.
- 35 Spermatid; axial filament curving about nucleus; segregation of nuclear material.
- 36, a Spermatid. Rupture of nucleus.
- 36, b, c Spermatids (fresh material); cell membrane dissolved; filament curved about nucleus; mitochondria beginning to clump on filament.
- 37 Spermatid. Centriole and rodlet touching nucleus; nucleus ruptured.
- 38, a Spermatid. Chromatin contracted into cup; mitochondria clumping on filament.
- 38, b Same, looking down into cup.
- 38, c Same, looking at point of contact of rodlet with nucleus from anterior end of cell.
- 38, d Same, viewing convex surface of cup.
- 38, e Same, fresh cell.
- 39 Spermatid; chromatin cup rounding out to form sphere.
- 40, a Spermatid; spherical sperm head; Golgi remnant lying in cytoplasm.
- 40, b Same, looking down on rodlet.
- 41, a Spermatid. Sperm head a flat disk.
- 41, b Same, in profile.
- 41, c Same, cross-section.
- 42, a Spermatid; elongating head; rodlet pressing into head.
- 42, b Same, in profile.
- 42, c Same, cross-section.
- 42, d Same (fresh material); centriole a rounded knob, lying in furrow.
- 43, a Spermatid. Rodlet sinking farther into head; keel at tip and lobes at base, prominent.
- 43, b Same, viewed at an angle.
- 43, c Same, from anterior.
- 43, d Same, in profile.
- 43, e Same, fresh material; cell membrane dissolving.

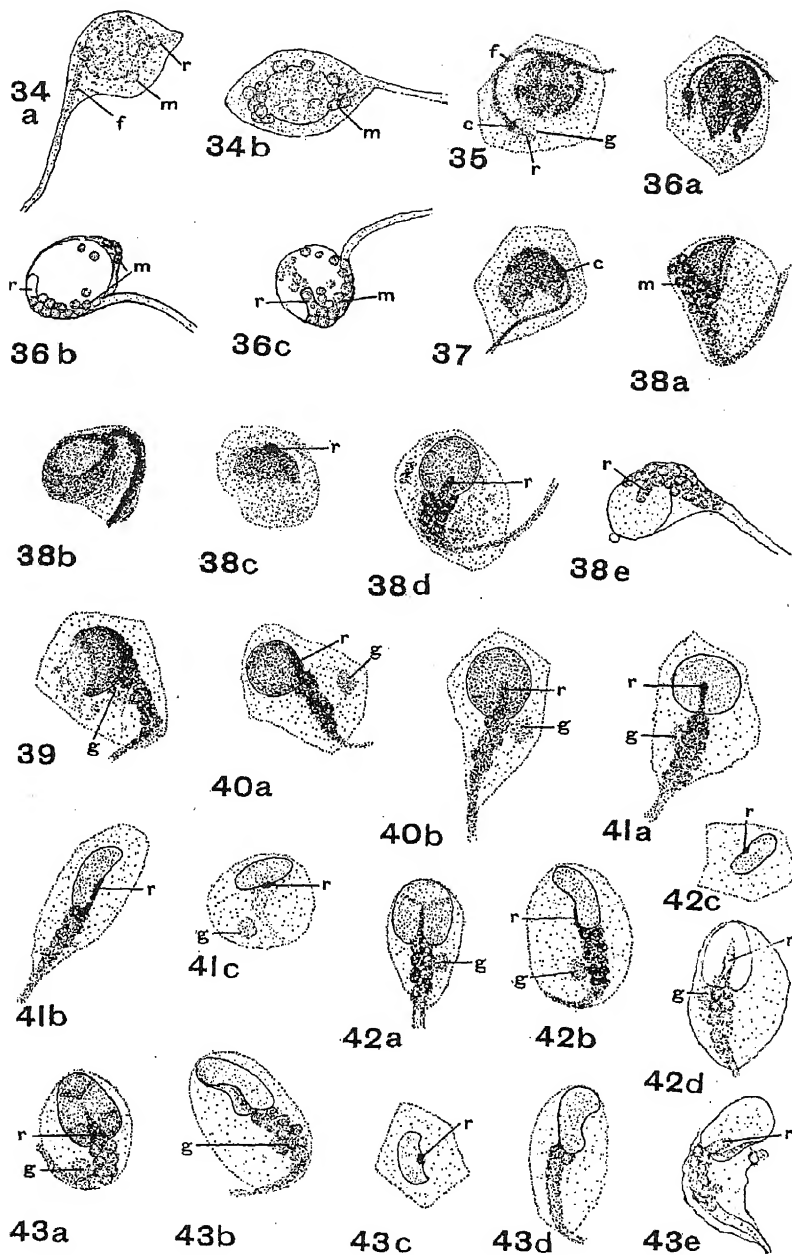


PLATE 5

EXPLANATION OF FIGURES

- 44, a Spermatid. 'Split-arrow' stage.
- 44, b Same.
- 44, c Same, cross-section. (Cytoplasm omitted.)
- 44, d Same, profile.
- 44, e Same, fresh material.
- 45, a Spermatid. Fissure closing.
- 45, b Same, in profile.
- 45, c Same, cross-section. (Cell membrane omitted.)
- 46, a Spermatid, ready to pierce cell membrane. (Membrane omitted.)
- 46, b Same, in profile. (Cell membrane omitted.)
- 46, c Same, cross-section. (Cell membrane omitted.)
- 46, d Same, fresh material; everting cell membrane.
- 46, e, f, g Same; stages in sloughing off residual cytoplasm.
- 47, a, b Fresh mature sperm.
- 47, c Same, showing irregular arrangement of mitochondria.

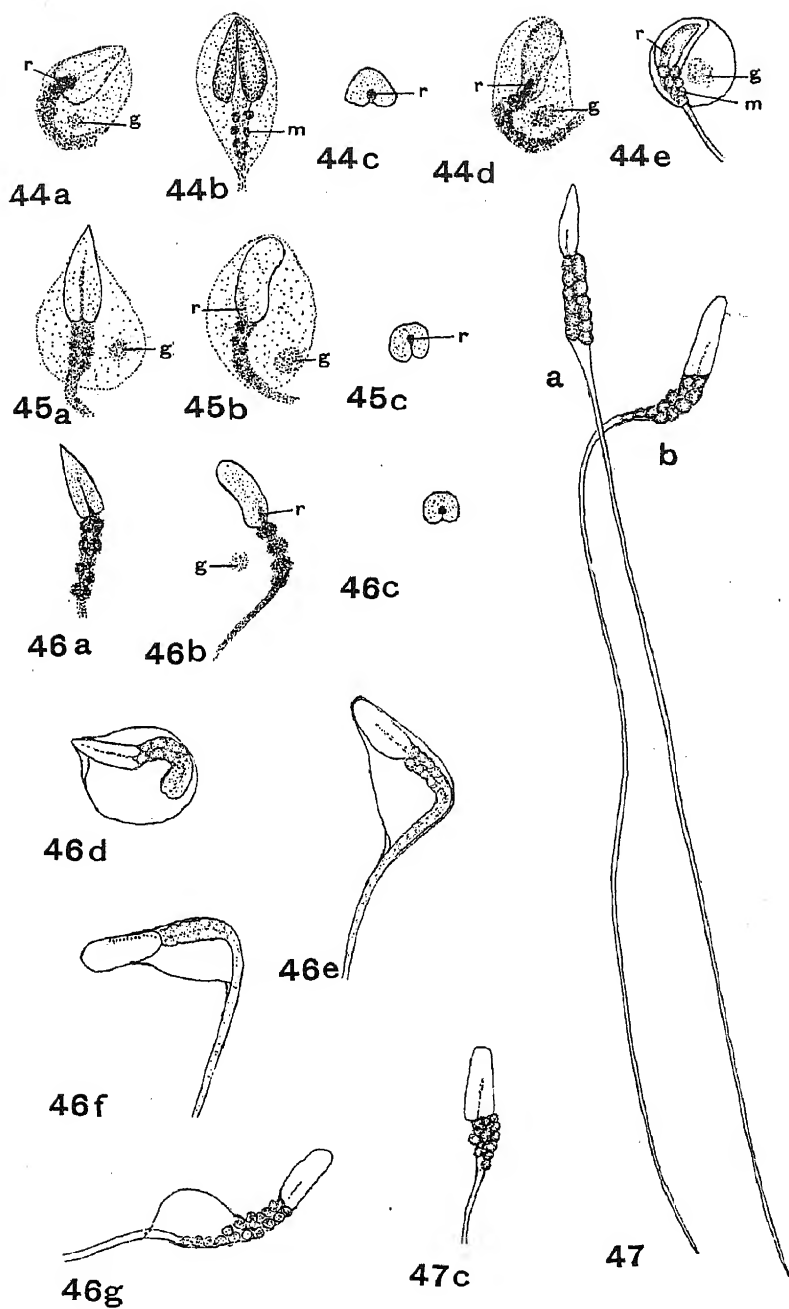
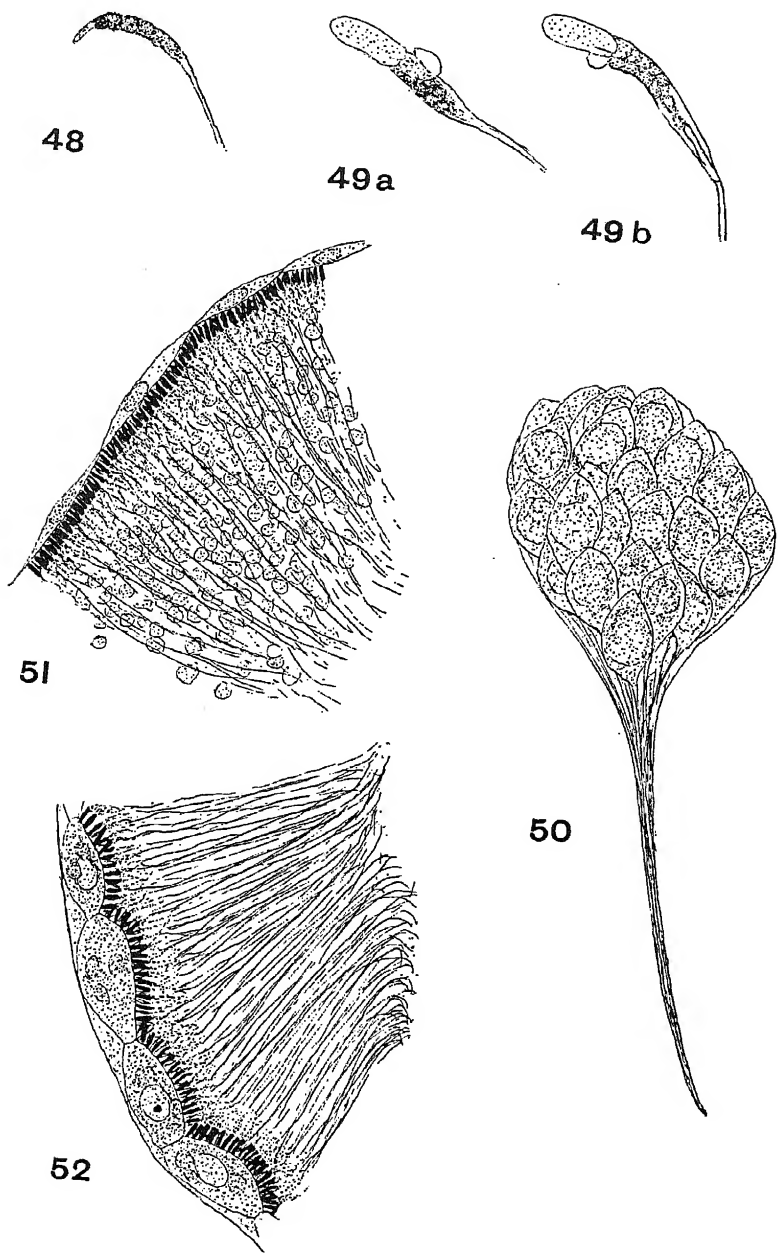


PLATE 6

EXPLANATION OF FIGURES

- 48 Rodlet, mitochondria, and axial filament; sperm head dissolved.
- 49, a, b Fresh sperm; clear vesicle on neck.
- 50 Bundle of fresh spermatids.
- 51 Sperm sloughing off residual cytoplasm; touching flat cells of cyst wall.
- 52 Sperm tightly pressed up against enlarged Sertoli cells.



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